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AGE AS A FACTOR INFLUENCING FATAL INFECTIONS OF HISTOMONIASIS IN CHICKENS

By

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INTRODUCTION

Histomoniasis (blackhead) is most prevalent and virulent in the turkey accounting for serious economic losses. It also occurs in chickens, quail, pheasants, guinea hens and grouse, and the author has seen a typical case of fatal blackhead in a peacock. Occasionally, serious outbreaks of the disease arise among flocks of chickens although the common fowl is generally considered to be the non-susceptible carrier host. Hungerford (1937) reported such an epidemic in New South Wales which killed 200 of a flock of 640 birds: in another flock, 100 of 400 birds died. He also noted that chickens were more susceptible to fatal infections of blackhead after they had been vaccinated for fowl pox. Bayon and Bishop (1937) obtained cultures of *Histomonas* from the liver of a hen which died of blackhead. Kaupp (1922) noted the death of 42 of 43 Silver Spangled chicks about 5 weeks old. Eriksen (1925) reported losses from blackhead in Missouri chicken flocks which ranged from one bird in a group of 350 to more than 50 per cent. in two other flocks.

The high cost of young turkeys has been a deterrent in the prosecution of large scale experiments on the disease. In employing any other animal for experimental purposes it would be desirable to produce in that animal an infection of the disease as it is found in turkeys. Chickens, as noted above, are to a certain degree susceptible to fatal infections; furthermore, they are less expensive and relatively easy to manage in the laboratory. In the experiment to be described groups of birds of varying ages were employed and their susceptibility to fatal infections assessed in terms of age. Little work has been done on those factors which cause the virulent form of the disease in chickens and no previous work has been carried out on the possible effect of age.

EXPERIMENTAL PROCEDURE

Infected liver and caeca were recovered from a turkey moribund with histomoniasis. This material was minced with a pair of curved scissors and the mixture ground in a mortar. The tissue was then passed through a fine wire sieve and suspended in Ringer's solution. An injection of this thick suspension was made *per rectum* by means of a Pasteur pipette the end of which had been flamed smooth. Each chick received 0·5 c.c. of the suspension.

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A preliminary enema was unnecessary since the injection readily "took" if the birds were starved for 18 hours previously. A practice was also made of binding the birds' legs with rubber bands and suspending them upside down for 5 minutes after rectal injection in order to ensure complete retention of the fluid.

The age group of the chickens used and the number in each group is shown in Table I. Six 5-week old turkeys were similarly treated to provide a "maximum infectivity" control and to serve for comparison with the chickens as to the course and the pathology of the disease. Another control group consisted of normal chickens which were segregated although they lived under conditions similar to those of the experimental groups. All birds were fed a balanced mash diet with the addition of 20 per cent. fishmeal and were reared on wire floors in glass-enclosed brooder cages. Drinking and feeding troughs were placed inside the cages and it was therefore possible for the birds of each group to ingest each other's droppings. These somewhat unhygienic conditions were maintained in order to determine what the maximum infective rate might be for a group that had ample opportunity for reinfection. The death rate of a group was the only criterion of that group's susceptibility to the disease. Diagnosis of histomoniasis was made on the basis of overt, gross abnormalities of the liver and caeca. When only caecal lesions were evident the presence of *Histomonas* observed in stained sections was used to confirm the diagnosis.

TABLE I

Group	*No. in group*	*Age (days)*	*Experimental or Control*
A	15	6	experimental
B	12	13	experimental
C	14	21	experimental
D	10	34	experimental
E	15	6	control (chicken)
F	6	35	"maximum infectivity" control (turkey)

RESULTS

The results obtained are listed in Tables II and III. The percentage mortality for all experimental groups of chickens was found to be lower that that of the group of infected turkeys. The highest mortality occurred in the group of 21-day old chickens. It was initially expected that the youngest birds would prove to be the most susceptible, but this, in so far as percentage mortality is concerned, was not the case. It is possible that, because of the frequent caecal evacuations of these young birds, the histomonads were voided before they had a chance to become established in the

caeca. In one sense, however, six-day old chicks did appear most susceptible in that they exhibited the shortest period to death (5 to 6 days) and succumbed to caecal infection only, without showing pathological changes in the liver. The caeca of these birds did not show the typical extensive necrotic lesions but rather exhibited many haemorrhagic petechiae. In these cases the mid-caecal regions were inflamed and superficially did not differ grossly in appearance from that found in coccidiosis; however, microscopical examination of these tissues demonstrated only *Histomonas*, and oocysts were never seen in the faecal content. The 13- and 21-day old groups gave the highest death rate; this death rate dropped sharply for the 34-day old group. The gross liver pathology of the 13- and 21-day old birds varied from widely spread, very numerous miliary lesions to patchy, coalescing necrotic areas. The condition of the caeca was similar to that found in the acute type of disease in turkeys. The 34-day old birds which exhibited a mortality rate of 30 per cent. were the most resistant. Deaths in this instance did not occur until the 12th and 15th day after inoculation—a period that more nearly coincided with the pre-death period of the group of turkeys of similar age. The gross pathology of this group was more like that found in the turkeys than that of the younger chickens. The liver lesions were typically round and circumscribed while the caeca were highly necrotic and cored. The bird dying on the 15th day showed large, pale yellow liver lesions and the gall bladder was highly distended. The affected caecum, although possessing an unhealthy looking white colour and being somewhat enlarged, showed marked evidence of repair. In all other birds which succumbed both caeca were affected. All uninfected controls remained normal and healthy.

Portions of liver and caecum of the bird of the 34-day old group dying on the 15th day after inoculation were minced together and suspended in Ringer's solution. This infected material was then injected *per rectum* into six 15-day old chicks. None of these chickens ever showed symptoms of the disease though the reason for this is obscure. A possible explanation may be that passage through chickens diminishes the parasite's ability to cause infection and if this is so, the maintenance of strains by means of successive tissue subpassage might not be feasible.

Considering the high cost of turkeys and the fact that they are not generally available during the autumn and winter months, chickens would be useful for drug screening experiments. Although the mortality in chickens did not attain the high level experienced in turkeys, the chickens of 21 days old used in this experiment did give a mortality rate of 71 per cent. which is certainly sufficiently high to be of use for drug screening if subsequent work should confirm the special susceptibility of this age group.

TABLE II

Day	*Group C*	*No. Dead*	*Pathology*
5	A	4	Extensive haemorrhagic petechiae of both caeca. Caeca dilated, have friable plug.
	B	3	Conditions like those of Group A.
6	A	4	Caecal necrosis and plug. Early miliary lesions of liver.
	C	3	Conditions similar to those of Group A.
7	No deaths		
8	B	1	Caecal necrosis and plug. Miliary lesions of liver.
	C	2	Conditions similar to those of Group B.
9	B	1	Caecal necrosis and plug. Liver shows coalescing miliary lesions.
	C	1	Conditions similar to those of Group B.
10	B	2	Caecal lesions and plug. Extensive, confluent patchy lesions.
	C	1	Caecal lesions and plug. Necrotic, irregular patchy liver lesions some 2 mm. in diameter.
11	A	1	Caecal lesions and plug. Miliary lesions of liver.
12	D	2	Caecal lesions and plug. Liver possesses circumscribed lesions as found in turkeys.
	C	1	Caecal lesions and plug. Patchy lesions of liver.
13	C	1	Conditions similar to Group C, Day 12.
14	B	1	Caecal lesions and plug. Faint irregular liver lesions.
	C	1	Caecal lesions and plug. Faint irregular liver lesions.
15	D	1	One caecum somewhat necrotic, shows signs of repair. Liver lesions 5mm. in diameter, round or oval shaped, yellowish in colour.

TABLE III

Group	Age (Days)	No. in Group	No. Died	% Mortality	Incidence of mortality on days following inoculation										
					5	6	7	8	9	10	11	12	13	14	15
A	6	15	9	60	4	4					1				
B	13	12	8	66⅔	3			1	1	2				1	
C	21	14	10	71		3		2	1	1		1	1	1	
D	34	10	3	30								2			1
E (control)	6	15	0	0											
F (turkey)	35	6	6	100						1	2	2		1	

SUMMARY

Chickens of various ages were inoculated *per rectum* with a suspension of liver and caecal material from a turkey affected with histomoniasis. It was found that susceptibility to infection was dependent upon the age of the chickens.

The highest mortality (71 per cent.) occurred in a group of birds 21 days old and the lowest (30 per cent.) in a group 34 days old.

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A SELECTIVE MEDIUM FOR THE ISOLATION OF ORGANISMS OF THE SALMONELLA GROUP

By

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INTRODUCTION

Many selective media have been described for isolating pathogenic intestinal organisms. Those in current use have proved of limited value in the recovery of pathogenic gram-negative bacteria, but reasearch for a better medium is still being carried out (Fredericq, 1947; Jeter and Wynne, 1949.) The medium suitable for routine diagnosis is one which gives the maximum number of positive results after incubation for 24 hours, sharply differentiates pathogenic from non-pathogenic intestinal organisms, and inhibits the latter to an extent which prevents them from overgrowing the former.

One of the greatest difficulties was to inhibit the growth of normal intestinal bacteria, particularly *Bact. coli* and *Proteus,* and to obtain the maximum growth of the Salmonella or Shigella groups since it has been observed by Halbert (1948) that lactose-fermenting faecal micro-organisms possess a high degree of inhibiting activity against non-lactose fermenting organisms. In order to suppress the growth of *Bact. coli* and other non-pathogenic intestinal organisms, brilliant green or malachite green has been used by some workers (Browning, Gilmour and Mackie, 1913), and the media designed by Wilson and Blair (1931) and by Jones (1936) incorporate one of these dyes. None of these media, however, is entirely satisfactory for all species of the Salmonella group, since brilliant green, in addition to its toxic action on *Bact. coli,* may also completely suppress some Salmonella. This disadvantage has been overcome by the use of a medium containing milk, which reduces the toxicity of the dye for pathogenic organisms, but not for coliform bacilli.

In order to distinguish the lactose fermenters, neutral red and acid fuchsin were added. The latter was incorporated to intensify the colouration of lactose-fermenting organisms since Stearn and Stearn (1924) have shown that colonies producing acid tend to be coloured by acid dyes.

PREPARATION OF THE MEDIUM

After consideration of these basic data the following medium was prepared:

3 per cent. nutrient agar	700·0 ml.
Sodium taurocholate	5·0 g.
Neutral red	0·05 g.
Brilliant green	0·5 g.
Acid fuchsin	0·5 g.
Boiled milk	300·0 ml.

The basic medium is 3 per cent. nutrient agar containing 0·5 per cent. of sodium taurocholate adjusted to pH 7·2 and kept in 100 ml. bottles each containing 70 ml. of medium. When preparing plates, melt the agar and to each 70 ml. add 0·5 ml. of 10 per cent. watery solution of acid fuchsin, 0·05 ml. of 10 per cent. watery solution of neutral red, 0·5 ml. of 10 per cent. alcoholic solution of Brilliant green, and 30 ml. of milk. All components should be sterilized before use and mixed thoroughly before pouring into Petri plates. The medium is blue.

RESULTS WITH THE NEW MEDIUM

Colonies of lactose-fermenting organisms have a deep indigo colour which does not diffuse into the medium, while non-lactose fermenters are white. Pseudomonas, if present, grows well, but can be easily recognised by a slightly yellowish-green discolouration and a "halo" round the colonies. Proteus is partially, and gram-positive bacteria are completely suppressed. Paracolon colonies are white and may at first be picked off as suspected Salmonella, but after 48 hours or even earlier they develop a pinkish centre. Slide agglutination with colonies picked directly from the plate is satisfactory as most of the colonies are in a smooth phase and give uniform suspensions. The organisms of the dysentery group are either partially or markedly inhibited and, in order to obtain a good growth, the strength of the dye should be reduced to 1 : 5,000.

The effect of this medium on the growth of small numbers of various organisms of intestinal origin which were examined may be summarised as follows:

Organisms not inhibited: *S. enteritidis, S. typhi murium, S. thompson, S. pullorum, S. gallinarum, Paracoli* and *Pseudomonas pyocyanea.*

Organisms partially inhibited: *Sh. newcastle, Sh. sonnei, Proteus vulgaris.*

Organisms markedly inhibited: *Bact. coli, Sh. shigae, Sh. flexneri,* gram-positive organisms.

Comparison of the new medium with sodium desoxycholate medium on the growth of mixed cultures.

Suspensions of 24 hour cultures of *S. enteritidis* and *Bact. coli* of similar opacity were mixed in equal proportions and standard loopfuls of the mixtures were plated on the new medium and on the sodium desoxycholate medium modified by Hynes (1942). After 24 hours incubation at 37° C., colony counts were made. The results are recorded in Table I.

Comparison of the new medium, blood agar medium and sodium desoxycholate medium on the growth of S. enteritidis.

In order to ascertain whether the new medium gave the maximum growth of *S. enteritidis,* various dilutions of *S. enteritidis*

TABLE I

COLONY COUNTS OF MIXTURES OF *S. enteritidis* AND *B. coli.*

Mixtures of B. coli *and* S. enteritidis *in equal proportion.*		*Numbers of colonies after 24 hours on the new medium.*	*Number of colonies after 24 hours on the sodium desoxycholate medium.*
Coli	10^{-4}	0	over 100
Salmonella	10^{-4}	more than 100	56
Coli	10^{-6}	0	over 100
Salmonella	10^{-6}	76	23
Coli	10^{-8}	0	over 100
Salmonella	10^{-8}	27	9

were prepared in saline and a standard loopful inoculated on the new medium, on desoxycholate medium and on blood agar respectively. After 24 hours incubation colonies were counted. The results are recorded in Table II.

TABLE II

COMPARATIVE STUDIES WITH NEW MEDIUM, BLOOD AGAR AND SODIUM DESOXYCHOLATE MEDIUM.

Dilutions of S. enteritidis	*Number of colonies*		
	New medium	*Blood agar*	*Sodium desoxycholate*
10^{-2}	numerous	numerous	numerous
10^{-6}	47	58	27
10^{-8}	15	17	5
10^{-9}	0	2	0

The results showed that the new medium gave nearly as many colonies as blood agar, and better results than the sodium desoxycholate medium.

Detection of organisms in faeces.

Similar experiments were performed to find the minimal dilutions of Salmonella organisms which could be detected in faeces. No significant difference was found between the new medium and the sodium desoxycholate medium in isolating Salmonella organisms from faeces. Contaminants develop more readily on the sodium desoxycholate medium than on the new medium; moreover the latter gave much sharper differentiation between Salmonella and contaminants. The new medium has been used with good results in routine work for the isolation of *S. pullorum* and *S. gallinarum* from infected birds.

DISCUSSION

The medium which is described in this paper appears to be of value for the isolation of all members of the Salmonella group. It combines two features which are essential for a good selective medium: (*a*) a sharp differentiation between the colonies of pathogenic and non-pathogenic intestinal organisms and (*b*) selective inhibition.

A comparison made between the new medium and sodium desoxycholate medium showed that the former is superior since it differentiated much more sharply the colonies of the Salmonella group from those of normal intestinal bacteria and inhibited *Bact. coli* and *Proteus* to a greater extent. It also yielded a much larger number of positive results than sodium desoxycholate medium. Slide agglutination with colonies picked directly from the new medium gave satisfactory results, though various workers have reported that serological identification is difficult in the case of suspensions prepared from growth on media containing dyes. The dysentery bacteria were found to be more sensitive than the Salmonella group to those concentrations of the dyes which must be used to inhibit the non-pathogenic intestinal organisms to a reasonable extent.

SUMMARY

A new medium has been devised which sharply differentiates Salmonella from non-pathogenic intestinal organisms and prevents the latter from overgrowing the pathogens. More clear cut results were obtained with the new medium than with sodium desoxycholate medium.

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EXPERIMENTAL "ALLERGIC" ENCEPHALITIS: ATTEMPTS TO PRODUCE THE DISEASE IN SHEEP AND GOATS

By

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INTRODUCTION

The possibility that injections of normal brain and cord might be pathogenic to the nervous system, was envisaged decades ago, when fatal neuroparalysis was observed on rare occasions to follow anti-rabic vaccination in man. Ideas that such cases might be due to the "virus fixe" or to some latent virus stimulated by the vaccine took a long time to dissipate (see van Rooyen and Rhodes, 1948). It has taken much longer to establish that the rabbit nervous tissue of the vaccine might be responsible for such post-vaccinal encephalitis. All the earlier work was reviewed by Hurst (1932) who concluded there was no evidence then to make decisive commitment in any direction. The first progressive step thereafter was the observations of Rivers, Sprunt and Berry (1933), Rivers and Schwenkter (1935), that demyelinating encephalopathy was produced in some monkeys by normal sterile rabbit brain; after multiple inoculations a protracted latent period ensued before the onset of the paralysis. Inconstant results were obtained by later workers some of whom used different species of experimental animals including sheep* (see Kolb, 1950, for a tabulated comparison of these results). A much greater impetus to further study arose from the experiments of Kabat, Wolf, and Bezer (1947) and Morgan (1947); by injecting normal brain with adjuvants they produced a demyelinating disease in monkeys by fewer injections and with shorter latent periods. From these studies have stemmed the many other investigations which have amplified the original conceptions. This may lead to the identification of a chemical agent in normal brain which produces so-called "experimental allergic encephalitis" in most species tried, and follows the injection if either homologous or heterologous brain tissue is used. The mechanism involved still remains to be proved as a true allergic one. This chemical factor which would appear to be residual in the phosphatide fraction of brain tissue, is water soluble, resistant to autoclaving, freezing, alcohol, ether, formalin and ultra-sound. Bell, Habel and Wright (1949) showed that it can be removed from rabies vaccine by calcium acetate and that it is dialysable through a cellophane membrane. The work cul-

* The author in 1935 injected suspensions of normal sterile rabbit brain to sheep and lambs, thrice weekly, for over a year with completely negative results. In view of what is known now this might have been related to the technique used then in preparing the suspensions.

minating in these developments was reviewed by Peers (1950), Kolb (1950) and Innes (1950a) and few of the individual experimental works need be cited here. The most recent one is that by Lumsden (1949b) which also contains many references. The review of Peers is mainly concerned with a comparison of the experimental lesions with those in human post-vaccinal encephalitis, while that by Kolb deals with the implications of the experimental work towards demyelinating diseases in general. Other articles (e.g. Wolf, Kabat and Bezer, 1947) compared allergic encephalitis with disseminated sclerosis, post-vaccinal encephalitis (after rabies and small-pox vaccination), post-exanthem encephalitides and Schilder's disease. This is of little direct concern here, but the opinions of Lumsden and Peers are to the effect that the close resemblance of this experimental disease to human post-vaccinal encephalitis must be significant although it is dubious if any analogy with disseminated sclerosis is justified. Further, it is manifest that the links beween the experimental condition and the naturally occurring encephalitides of man and animals, remain to be forged.

Experiments with domesticated animals were undertaken for various reasons. When new techniques are discovered for the experimental study of disease processes there is a logical tendency to examine the relative values of different laboratory animals. In the field of allergic encephalitis, on account of expense in using monkeys, guinea pigs have now become the species of choice while mice might also be useful in assaying tissue fractions for the presence of the encephalitogenic agent (Olitsky and Yager, 1949). There are also advantages—not only to comparative pathology—when projects are extended to include domesticated animals. Fundamental differences in pathology may be discovered when the same aetiological agent is used in varied species, and diverse types of causal factors may produce a comparable histological reaction in the nervous system, (cf. the disparate noxae which produce experimental demyelination). As the pathogenesis of swayback of lambs is unsolved, a comparison of this ovine demyelinating disease with allergic encephalitis was deemed of possible value. Secondly, the post-infective encephalitides form an important group of demyelinating diseases of man; parallel entities in domesticated animals have not been proved to follow in the wake of variola or any other exanthematous virus infection while the only post-vaccinal encephalitis recorded in animals has been that in dogs* after anti-rabic vaccination. In Britain, however, the use

* Even in the dog, it is still unproven whether there is any encephalitis which can be placed in the *post-infective* category for there have been far too many loose assumptions by veterinarians along this line, (c.f. common usage of terms such as post-distemper encephalitis). Those forms of encephalitis, which have been established to have any causal connection with a virus, would seem on available evidence to be due to invasion of the nervous system by a virus which has a primary effect on some visceral tissue, i.e., they are not truly post-infective.

of louping-ill vaccine (made from infected sheep brain) in sheep might, theoretically at least, provide the mechanism whereby such post-vaccinal cases could occur. If allergic encephalitis was capable of being produced in this latter species it might give a clearer picture of what might be expected in the clinical course and pathology of ovine post-vaccinal disease. In this connection it was shown earlier that louping-ill vaccine was as potent as samples of normal sheep or rabbit brain in producing allergic encephalitis in guinea pigs.

EXPERIMENTS IN GOATS AND SHEEP*

Materials and Methods.

The brain emulsions for injection were made in the way practised by investigators since the technique was devised by Kabat *et al* and Morgan. Frozen sheep brain (20 per cent. aqueous suspension) was mixed in a Waring Blendor in equal quantities with a mineral oil mixture containing 1 mg. heat killed and dried *Mycobact. tuberculosis,* per cc., and the emulsifier (Arlacel A), made by Atlas Powder Co., Wilmington, Delaware. In the first experiment 8 adult sheep were used (18 months to 2 years of age); they were given 5 intramuscular injections of 5 cc. over two weeks. In the second experiment 9 lambs (a few weeks old) were used; the emulsion was made from fresh sheep brain and a single injection was given into the nape of the neck. At the same time as the first experiment 8 adult goats were inoculated with a series of five injections. The emulsions used were potent in producing allergic encephalitis in guinea pigs. All animals were observed at regular intervals for neurological manifestations, and after death were subjected to autopsy. The usual selective histological techniques were applied in the preparations of the slides of the nervous system. The results of these examinations, with other details of the animals, are apparent from the descriptions below.

GOATS

Four of the eight goats survived 70, 86, 151, and 183 days after the first injection; during life none of them showed any sign of paralysis. The one surviving 151 days showed a tendency to scissors-gait of the forelimbs and tremors around the 126th day, but these signs never progressed and the animal was finally killed. The other four animals died from other causes within 30 days after the first injection. Numerous sections of all levels of the nervous system of all 8 goats were made; there was no evidence of any lesions remotely resembling those of allergic encephalitis as seen in monkeys, guinea pigs, dogs, and—as will be shown—in sheep.

* Other work using dogs was carried out at the same time and will be reported separately.

SHEEP

Clinical findings.

In the first experiment with adult sheep there were four deaths preceded by loss of condition due to other causes in 12, 15, 16 and 32 days, but there were no neurological signs noted during life and no lesions were ultimately found in the nervous system. In the four remaining adult sheep, in which pathological lesions were found in the nervous system after death, a notable feature was the variable, but lengthy, latent period before the onset of signs. Death occurred in 53, 74, 132, and 151 days respectively. In the first three the course of the disease, once it began, was relatively rapid (6 to 9 days); gradual loss of condition was noted before initial signs such as coarse tremors of the head, neck and limbs; later in all the sheep there was a tendency to stumble and sway on turning with difficulty in rising and progressive spastic paralysis of the extremities. Finally the animals lay on their side (one for 2 days), were unable to rise and were then killed. The fourth animal of this group showed initially a high-stepping gait, head tremors, swaying and marked fatigue around the 95th day, but these signs did not progress immediately; it remained alive for another 56 days and during this time there may have been remissions for at times it appeared better; at 120 days it developed a scissors-gait and 7 days before death ataxia was most marked; the sheep then wobbled at every turn and when forced to move knuckled over and walked almost on its "knees". Any defect of vision was doubtful but none of these four sheep was totally blind.

In 6 out of 9 lambs in the second experiment nervous lesions were demonstrated after autopsy. There was a similar variability in the latent period (40 to 118 days) and the signs were like those already described, viz., early hind leg stiffness, loss of condition, tremors and later inability to trot and turn quickly. In two at least the early symptoms did not progress markedly and these were killed at 118 days. In the 3 lambs in which active disease was not subsequently found at autopsy there was some initial stiffness and body condition was never good. At no time were there signs of any progressive changes attributable to involvement of the central nervous system, and they were killed at 72, 73 and 118 days.

The signs in affected sheep or lambs were thus comparable to those seen in other species, viz., hypotonia, asthenia, tremors, ataxia and spastic paralysis progressing to immobility when the disease was fully developed. Constitutional signs were absent. In some it appeared the damage must have been so slight that recovery was possible. The length of the latent period was more marked than that apparent from the records of the workers using guinea pigs, and in this respect was more like the history of the monkeys in the very early work of Rivers *et al* (1933).

Pathology.

The salient features of the nervous lesions in this ovine disease

need only be considered, for detailed descriptions of the pathology of allergic encephalitis in other species are readily available.*

No characteristic *macroscopic* changes were present in the nervous system of any positive case, and certainly none such as those haemorrhagic and malacic-like patches described and illustrated by Wolf *et al* (1947) in the monkey, and which have also been seen personally in some experimental dogs.

The degree of change was independent of survival (or age) of the sheep, for the lesions were as severe in kind and as widespread in distribution in one case (53 days survival) as in others which lived beyond 100 days.

There were no thrombi, no perivascular haemorrhages, no necrosis of vessel walls and no notable swelling of the vascular endothelium. The neurones of the entire grey matter escaped severe damage. Meningeal infiltrations, which were absent in only 3 cases, were composed of lymphocytes and mononuclears; polymorphonuclears were rarely present, and eosinophils and plasma cells never seen. The meningitis, when present, was equally intense in the spinal leptomeninx (Fig. 1) as on the convexity and base of the brain, and was always more marked in the depths of the sulci rather than on the summits of gyri. In three cases the meningeal lesions were more striking than those in the nervous substance.

When there was dissemination of the disease process hardly a part of the central nervous system escaped even in moderate cases. Lesions would thus be present bilaterally, without being symmetrical, in the frontal and occipital lobes, corpus callosum, hippocampus, basal ganglia, capsules, mid-brain, pons, cerebellar white matter, medulla and cord. The white matter was attacked much more than the grey, but lesions were frequently seen in the latter in the mid-brain, basal ganglia and spinal cord, and often adjacent to normal neurones (Fig. 6); they were hardly ever found in the cerebral and cerebellar cortex. Periventricular regions were not particularly affected. Although when lesions were present, there was no strict anatomical selectivity, it seemed that the pons, brachium pontis, cerebellar white matter and cord were often sites of severe damage. In the cord the process must have been widespread for usually only two levels of cord (about C.6-T.1 and about T.12-L1) were taken for sections and disease was present to some degree at both levels in most animals.

In most animals the process was not a selective demyelination but was essentially a non-haemorrhagic perivascular inflammation in which veins and venules (rarely arterioles) were involved (Figs. 1 and 2). Cellular cuffs and sleeves of various densities were pre-

* See Farraro and Jervis (1940), Wolf *et al* (1947), Morgan (1947), Ferraro and Cazullo (1948) in monkeys; Jervis and Koprowski (1949), Lumsden (1949, a) and Alvord and Stephenson (1950) in guinea pigs; Olitsky and Yager (1949) in mice.

sent which might be restricted to the wall and perivascular space (Fig. 3), or might spread out to adjoining nervous substance to form a small focus. Lymphocytes predominated along with cells proliferated from the vascular adventitia (c.f. Lumsden, 1949a); polymorphonuclears and plasma cells were rarely, and eosinophils never, seen. In lesions of this grade glial reaction around the involved vessels was absent or slight (Fig. 3); *gemästete Glia* were absent, rod cells formed minute collections and astrocytic nuclei were swollen, but there was no obvious increase in macroglia or of glial fibrils. Myelin stains of such areas showed that any suggestion of myelin loss might be an illusion due to the pallor created by the myelin sheaths being pushed aside by the swollen perivascular cuffs or the spread out focus (c.f. Fig. 5). Bielschowsky preparations showed that the axis cylinders were nearly always intact close up to a perivascular focus, and in the spinal cord this was quite notable (Fig. 6). In the cord the lesions seemed to extend radially from the grey matter along the septi towards involved meninges (Fig. 1).

Small granulomatous foci were seen in some animals: some might have arisen by confluence of more minute lesions and all were not clearly related to vessels. These foci were mainly accumulations of epithelioid-like cells and lymphocytes; no eosinophils or true giant cells were found.

In yet other areas of the nervous system (sometimes of the same animal but not constant for every sheep) the perivascular infiltrations were surrounded by irregular zones of rarefaction which seemed to have been formed by confluence of smaller lesions (Fig. 4). In such foci there was degeneration and loss of myelin and the axis cylinders also tended to swell, bead and break up although they were more persistent (Fig. 7). This myelinoclasis never proceeded to malacia or cyst formation and was never evident in the fresh brain to the naked eye as gelatinous foci. The contours of such areas were always hazy and when stained by Nissl and by haematoxylin and eosin, it was manifest that *gemästete Glia* were not prominently involved; this was confirmed in frozen sections stained with oil-red-O in which the lipoid-containing-phagocytes were very scanty. Rod forms were numerous, but there were no eosinophils or giant cells. Swelling of astrocytic nuclei was a possible, but not a distinctly constant, feature and Hortega's silver carbonate method on frozen sections and the phospho-tungstic acid method on paraffin sections did not reveal any pronounced accompanying increase in fibrils. Glial plaques were not found. Areas of myelin rarefaction, when present, were found in various parts of the brain and spinal cord, and in the latter situation they often bordered the ventral median fissure. Lesions were sometimes found in peripheral nerves (small strips from axillary and sciatic plexuses) as cellular infiltrations of lymphocytes and plasma cells in the endoneurium and perineurium. It was uncertain how

much importance could be attached to this finding, for they might have been caused by a local reaction consequent to the emulsions injected tracking along fascial planes to nerves.

DISCUSSION

The inability to produce encephalitis in goats with the same emulsion used so successfully in sheep is peculiar and corroborates the finding of Lumsden (1949, b). The same author (1949, a) indicated that certain species were refractory to injections of normal brain emulsions. For example, rats were resistant to guinea pig brain and *vice versa* and it is difficult to produce the disease using mouse brain as antigen. This species resistance remains to be confirmed and its basis explained.

The production of experimental encephalitis in sheep does not elucidate any further the crux of the whole problem. It might however indicate, if further work was desirable with animals larger than the laboratory species, that sheep might be as useful as monkeys with the practical advantages of relative cheapness, ease of maintenance and handling. Multiple injections, as given to the adult sheep, were in effect unnecessary for they did not appear to influence the end result nor is the age of animal used of any practical importance. In sheep the disease was sometimes acute and fulminating; in a few animals the symptoms subsided with minor residual effects, and in one sheep at least there were suggestions of remissions and relapses.

A consideration of the ovine lesions indicates that they fall into line with those seen in other experimental species in which it has been claimed there is a fundamental pathological pattern. Six varieties of lesions have been described in monkeys and guinea pigs viz., meningitis, perivascular infiltrations, granulomatous foci, perivascular myelinoclasis, punched-out and irregular "moth-eaten" areas of demyelination and occasionally a marked haemorrhagic reaction. Of these, only the first three mentioned were more constantly seen in sheep while irregular foci of perivascular rarefaction were less common; the last two types of lesions were conspicuously absent. The massively acute polymorphonuclear and eosinophilic reaction in a morphological sense described in guinea pigs and monkeys was also absent. In some ways the ovine lesions were reminiscent of those seen in monkeys which survived for relatively long periods (see Wolf *et al,* 1948), but there were definitely no plaques of glial scarring found in the sheep. What factors contribute towards influencing the tempo of the process is unknown, but it is supposed that the time factor may influence the final pathological picture.

This experimental encephalitis in sheep is unique for it bears no comparison with any neurological entity known to occur naturally in any ruminant animal. It is totally unlike (a) the bilateral diffuse cerebral demyelination of swayback in lambs, (b) the purulent meningo-encephalitis of Listeria infections in

sheep and goats, (c) the subacute granulomatous lesions produced by Listeria in goats described by King (1940), (d) the non-demyelinating encephalitis in sheep due to Louping-ill virus infection, or (e) a peculiar necrotising encephalomyelitis of goats recently described by the author (1951, c). There has been no real post-vaccinal, or post- exanthem, encephalitis described in ruminants for further comparisons to be considered, and the above are the sole nervous diseases worthy of reference. The encephalitis produced might, however, appear to demonstrate by analogy that which might be expected to occur in post-vaccinal disease of sheep after louping-ill vaccination. Such cases, if they occurred, would be extremely rare. By analogy with these experiments, the facts that the nervous signs might be minor, transient and non-fatal, that a long latent period might follow after vaccination and that characteristic macroscopic changes might be absent are a few features which have caused such cases to be overlooked by interested observers.

No claim is made that the ovine lesions resemble those of human disseminated sclerosis in any way. The fact that negative results have attended all efforts in the past 50 years to lay bare the aetiology of this human disease might indicate that no potential avenue should be left unexplored (see Reese in "Multiple Sclerosis etc." (1950) and Schaltenbrand (1943)). Perhaps less intense insults to the nervous system of sheep such as smaller doses of normal brain emulsion might invoke a yet more chronic disease than that seen; as already indicated there were clinical signs of a remittent character in at least one case. These experiments might thus focus some attention on other possibilities. In previous experimental work relating to disseminated sclerosis it may be that the wrong types of animals have been used to identify or to rigidly exclude an infectious agent, or to produce any strict pathological counterpart.

No transmission work was done in these present sheep experiments to obviate the possibility that the brain emulsions contained an infectious micro-organism. This has been considered so fully by other workers, admittedly with other species, to leave no doubt as to the specificity of the reported result, viz., that the same disease, known in other species to be due to some chemical agent in normal brain tissue, was produced in adult sheep and lambs.

SUMMARY

In eight goats the injection of an emulsion of normal sheep brain with adjuvants did not produce an encephalitis.

In sheep, the same brain emulsions as used for the goats produced a characteristic neurological syndrome in 4 out of 8 adult animals and in 6 out of 9 lambs. Noteworthy clinical features included the long latent periods elapsing between injection and the onset of symptoms, the acute course in some animals, possible

recovery in a few "negative animals", and a relapsing disease in at least one positive case.

The lesions of ovine allergic encephalitis have some similarity to those seen in other species but acute haemorrhagic changes were not encountered. Areas of myelin rarefaction were occasionally found but selective perivascular, punched-out or diffuse foci of myelinoclasis were not observed—such as is seen in experimental allergic encephalitis in monkeys and in the natural variety of simian Schilders-like disease. (For review on the latter see Scherer (1944) and King and Meehan (1950).)

The disease does not resemble any type of naturally occurring encephalitis so far identified in a ruminant.

Sheep might be used with advantage in future attempts to produce an artificial counterpart of human disseminated sclerosis.

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ILLUSTRATIONS.

FIG. 1. *Spinal cord.* Adult sheep. (Neurological signs seen at 123 days, duration of illness 9 days until animal completely paralysed.) Ventral median fissure parallel to right margin ; disseminated foci in ventral columns of white matter ; some myelin rarefaction bordering along edge of fissure ; marked leptomeningeal infiltration.

H.E. × 75 (NIH 48446).

FIG. 2. *Spinal cord,* higher magnification of an area in Fig. 1 showing small granulomatous foci.

H.E. × 180.

FIG. 3. *Cerebellar white matter.* Adult sheep. (Neurological signs first noted 95th day with some remissions until death at 151 days.) Foci of lymphocytes and adventitial cells limited to perivascular spaces ; little or no glial reaction around.

H.E. × 103 (NIH 48669).

FIG. 4. *Cerebellar white matter.* Lamb. (Neurological signs with slow progressive weakness and pronounced about 14 days before death at 110 days.) Irregularly contoured area of perivascular rarefaction ; no haemorrhages ; few *gemastete Glia ;* moderate astrocytic and microglial response.

H.E. × 103 (NIH 50742).

FIG. 5. *Cerebellar white matter.* Same case as Fig. 4 but from another area which resembled the appearance depicted in Fig. 3. Perivascular pallor in myelin stain due to unstained cellular cuffs but not true selective demyelination. Frozen section.

Spielmeyer's method. × 103.

FIG. 6. *Spinal cord.* Same case as Fig. 3. Scattered lesions were present in the cord of this animal as in Fig. 1 and myelin preparations were like those in Fig. 5. Axis cylinders are intact close up to the sleeve of cellular infiltration stained intensely black and bordering grey and white matter.

Bielschowsky. × 103.

FIG. 7. *Spinal cord.* Same case as Fig. 1. Longitudinal section showing beading and fragmentation of axis cylinders in areas where there was rarefaction.

Bielschowsky. × 103.

FIG. 1

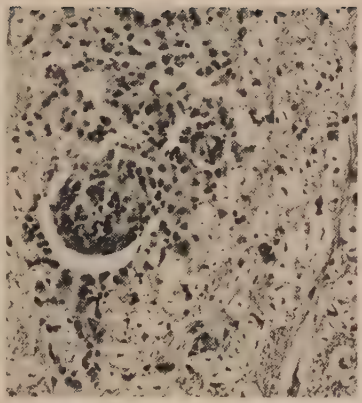

FIG. 2

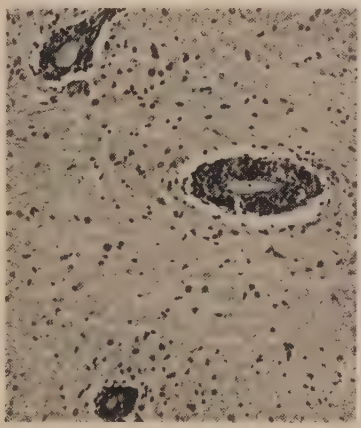

FIG. 3

FIG. 4

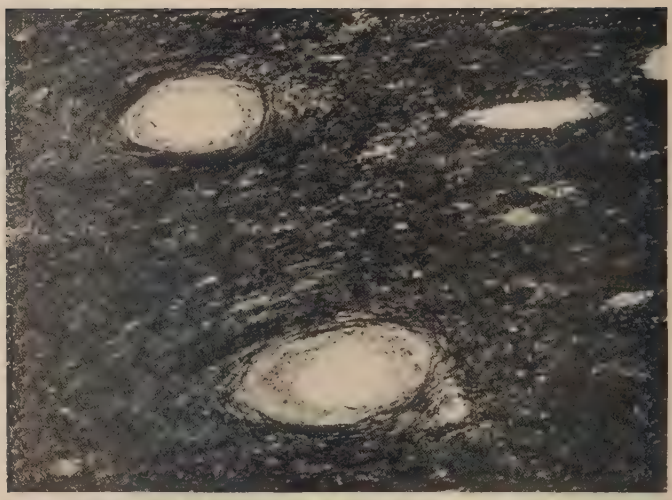

FIG. 5

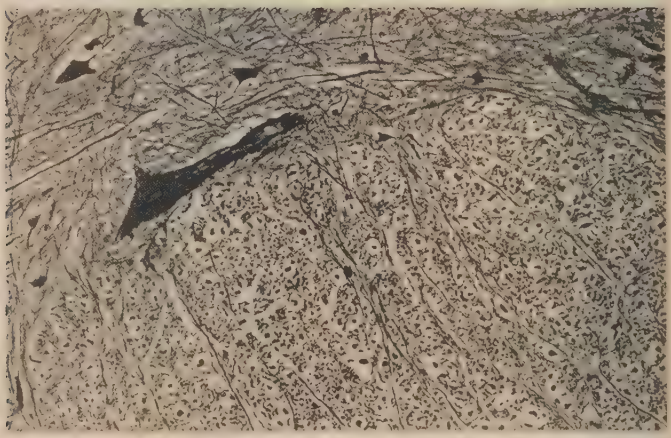

FIG. 6

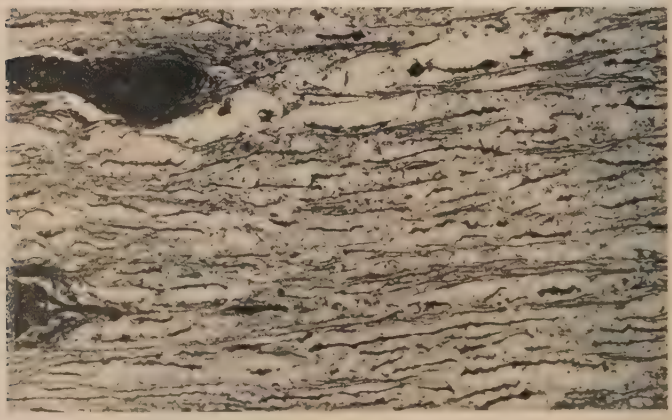

FIG. 7

INFECTIOUS ENTEROHEPATITIS (BLACKHEAD) IN TURKEYS: A STUDY OF THE PATHOLOGY OF THE ARTIFICIALLY INDUCED DISEASE

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INTRODUCTION

Since the original article by Theobald Smith (1895) in which he first described the condition now known as infectious enterohepatitis, studies on the pathogenesis of the disease have been attempted by a number of workers.

The first observations, recorded by Smith (1895), included a description of the gross changes in liver and caeca supplemented by microscopic examination of sections of tissue representing a relatively advanced stage of the disease. Infection was then considered to be due to an amoeba, although this protozoan was subsequently thought by Hadley (1916) to be trichomonad. Finally Tyzzer (1919), in a critical review of the subject, showed that the organism was a histomonad, and as a member of that species it now bears the name *Histomonas meleagridis*.

The site of primary invasion of the caecal wall was at first described by Smith as being located at the blind end of the caecum, but Hadley (1916, 1917) advanced the view that invasion was accomplished via the goblet cells in the crypts of Lieberkühn. Tyzzer (1919) also described invasion of the goblet cells but did not consider that primary lesions invariably arose in the crypts, whilst Jowett (1911) postulated a form of secondary invasion following initial damage by other parasites such as helminth worms or coccidia.

Most workers were agreed that subsequent development of the lesions in the caecal wall was characterised by a proliferation of small round cells accompanied by widespread oedema. Parasites were present intercellularly in the connective tissues and spread through to the muscular layer and thence to the serous coat. It was assumed that invasion of the liver took place via the blood stream, although there was no supporting evidence in favour of this beyond an observation by Smith (1895) in which he drew attention to the frequency with which liver lesions developed adjacent to blood vessels.

Further development of liver lesions was characterised by migration of the parasites through interstices in the tissue resulting in the appearance of a reticulum composed of degenerate parasites represented by vacuoles, and at this stage giant cells distended by parasites have been described by Jowett (1911) and Tyzzer (1919).

The most informative account of the morphological characteristics of the parasite throughout the course of the disease has been presented by Tyzzer (1919). In his opinion the protozoan presents three distinct phases which he described as invasive, vegetative and resistant. The invasive form was described as a small round or oval cell, 8-17μ with basophilic cytoplasm, which was seen on the periphery of early lesions,

in the goblet cells and epithelium of the caecum, and in between the liver cells. This cell had the features of a migratory parasite. The vegetative phase was non-motile, varying in size from 12-21μ, and possessed ring-like cytoplasmic inclusions which probably represented a degenerate form of the parasite. The resistant phase was characterised by a dense membrane, relatively small in size, from 5-11 μ, with a feebly pink, diffuse staining cytoplasm. Its nucleus was irregular in shape and the whole appearance suggested encystment. Multiplication of the parasites within the tissues probably took place by binary fission, and there was no evidence to suggest that reproduction occurred by sporulation, a view which was advanced by Hadley (1917).

Histological examination of organs from artificially infected turkeys was carried out by Tyzzer and Fabian (1920), but these were from birds in which the disease was relatively far advanced. The liver lesions resembled the appearances shown in spontaneous blackhead, but as infection was by subcutaneous inoculation there appear to have been no lesions in the caecum. The development of liver lesions, however, supports the view that infection spreads via the blood stream.

A further study of tissues from artificially infected chickens was conducted by Tyzzer (1936), rectal inoculation of cultures being used as a means of infection. Here it was observed that invasion of the caecal mucosa took place soon after inoculation and within 48 hours large numbers of protozoa were seen between the fundi of the glands. This invasion was accompanied by an intense infiltration of the submucosa by polymorphonuclear leucocytes, which was the chief characteristic of the initial stage. Subsequently the picture changed and with the multiplication of the histomonads there was extensive infiltration with monocytes which gradually checked the migration of the protozoa by confining them within the intercellular spaces. The organisms continued to divide but soon disappeared, the process being accompanied by oedema and increase of lymphoid cells. The initial reaction may have been due to bacteria as a caecal membrane was produced by the outpouring of fibrino-purulent exudate. No conclusion, however, was reached as to the exact mechanism of destruction of the parasite in the tissues.

In most cases material for the above studies was obtained from birds suffering from the naturally acquired disease. This imposed certain limitations on the scope of the work in view of the fact that poults were showing clinical symptoms before being subjected to post-mortem examination. Thus the disease was relatively advanced and studies of a histopathological nature could not reveal evidence of changes associated with the initial stages of the infection.

Previous work in these laboratories (Farmer and Stephenson, 1949) established a method of inducing the disease by artificial means and it seemed desirable to make use of this in an attempt to study the nature of the disease throughout its course, with particular reference to changes occurring in the initial stages.

METHODS

Experimental Birds. Forty 4 week old turkey poults from the same batch were placed in groups of three in large cages, in a room

maintained at a temperature of 65° F. The poults had been incubated and hatched on the premises. The breeding stock which produced these poults were part of the Division's flock. They were kept in folds which were moved daily. Latterly when clean ground was no longer available the breeding stock were kept in houses with intensive sun parlours raised off the ground. None of the breeding flock used to produce poults for the experiments suffered at any time from blackhead.

Mode of Infection. Thirty-six of these poults were infected by the method described by Farmer and Stephenson (1949) using the caeca from four experimentally infected poults, emulsified in 250 ml. of Ringer's solution.

Collection and Treatment of Material. The four uninfected birds were killed during the course of the experiment on the 1st, 4th, 8th and 12th days respectively, and served as normal controls. Of the remaining birds one group of three was killed on each consecutive day following the day of infection.

The bodies were opened with care immediately after killing and material for histological and bacteriological examinations was removed with as little delay as possible. This was generally achieved within five minutes of opening the carcase. Portions of the liver and caeca were first taken aseptically and ground up in Griffiths tubes for inoculation on to blood agar and McConkey plates.

Thin slices of liver, caeca, spleen and, when indicated, omentum, or mesentery were removed and fixed in Heidenhein's "Susa" solution. They were then dehydrated, cleared and embedded in paraffin wax in the normal way. Sections were cut at 6-8μ from each block and were stained by the following methods:—(*a*) Ehrlich's acid haematoxylin and aqueous eosin; (*b*) Heidenhein's iron haematoxylin (long technique) and Minchin's Lichtgrün; (*c*) Overnight in dilute Giemsa, followed by differentiation in 1 : 1000 acetic acid.

One caecum and one lobe of liver from each bird was preserved in Jore's solution for macroscopic observations.

Photomicrographs were recorded on Ilford thin film half-tone plates. Colour records were made on Kodak Ektachrome Type B sheet film.

RESULTS

Portions of the tissues were subjected to bacteriological examination in order to obviate the possibility that bacteria might be involved in the Blackhead complex. There was no evidence that bacteria played any part in the disease process. The specimens of liver throughout the series were almost free from bacteria, whilst in the caeca the predominant organisms were of the coliform group.

The results of the macroscopic and microscopic examinations

changed as the disease progressed as was to be expected, but in many cases there was some variation in the appearances observed in the organs of poults killed on the same day. These variations were probably due to the varying degree of natural resistance exhibited by individual birds. An account of these findings is presented in the following consecutive observations.

Control Poults

These birds were killed on days 1, 4, 8 and 12 in an effort to detect any histological change which might be misleading in our interpretation of material from diseased poults. The caeca exhibited a typical intestinal mucosa with no sign of ingested cellular material in the goblet cells or any marked cellularity of the sub-mucosa. When caecal contents was present it was not markedly cellular. The liver also showed a normal structure and, in view of subsequent findings in diseased birds, the absence of areas of small round cell infiltration was particularly noted.

Infected Poults

Day 1.

Caeca. There were no gross abnormalities in the caeca of poults killed on this day. On microscopic examination there was evidence of increased cellularity of the caecal contents. Debris lay in the constricted folds of the mucous membrane, and some of the crypts of Lieberkühn were distended with this type of material. In one section there were groups of pear-shaped protozoa in the contents of the lumen and there were also a number of similar cells adhering to or lying on the surface of the epithelium (Fig. 1). In the epithelial layer there were a number of acidophilic cells with densely staining, granular cytoplasmic inclusions. Similar cells were also observed in the connective tissue immediately adjacent to the epithelium.

Livers. There were no significant macroscopic or microscopic changes.

Day 2.

Caeca. The caeca showed some evidence of hyperaemia, but there was no evidence of macroscopic lesions. Microscopically the contents of the lumen were rich in protozoa, some of which were flagellated. In one section, protozoa were seen on the mucous membrane. In some places the mucous membrane stained poorly and it was loosened or detached from the underlying tissue. This detachment appeared to be associated with a pink homogeneous material, probably exudate, which lay between the mucous membrane and the underlying tissue. The exudate was most prominent at the tips of the villi. (Fig. 2). There was a commencing polymorphonuclear leucocytosis in the tissues near the mucous membrane, and some suggestion of an increase in the lymphoid tissue.

Livers. Macroscopic lesions were not yet observable. On microscopic examination of one section one small area of round cell infiltration was seen close to a blood vessel. All sections showed early changes of cloudy swelling.

Day 3.

Caeca. All caeca showed hyperaemia and marked distention, with slight thickening of the mucosa. One caecum contained a number of moderately sized haemorrhagic lesions. Histologically the caecal contents contained some red blood cells and a catarrhal exudate in the crypts contained protozoa. The exudate and detachment of the mucous membrane at the villus tips was well marked. The capillaries in the lamina propria were engorged. This congestion extended into the deeper layers of the organs as far as the muscular and serosal layers. There was an infiltration by inflammatory cells, mostly polymorphonuclear, of the submucous layer, muscularis mucosae and the adjacent areolar tissue. There was also an increase in lymphadenoid tissue (Fig. 3). Associated with the inflammatory reaction in the submucous tissue were small aggregations or "nests" of protozoa. In the centre of one blood vessel there was a protozoan, which appeared to be a histomonad (Fig. 4).

Livers. There were no visible lesions on naked eye examination. Microscopically, cloudy swelling and capillary congestion were present. There were a number of foci of small round cell infiltration (Fig. 5). In one of the foci there was a cell containing four nuclear fragments, and another cell had several dense basophilic granules arranged in a crescentic manner; these were possibly of protozoal origin. Some of the foci presented evidence of pyknosis of the cells.

Day 4.

Caeca. All caeca were distended and hyperaemic and one caecum showed numerous capillary haemorrhages, thickened mucosa and well defined greyish white lesions. This caecum in section presented a considerable degree of capillary congestion even in the muscular and serosal layers. The congestion was accompanied by haemorrhages in parts of the lamina under the mucous membrane. There were increased numbers of polymorphonuclear cells in the mucous membrane. The catarrhal exudate containing protozoa could still be seen in the crypts of Lieberkühn. Sections of caeca from the remaining poults showed little pathological change.

Livers. No gross changes were visible. Only one section contained a focus of round cell infiltration accompanied by polymorphonuclear leucocytes.

Day 5.

Caeca. All were enlarged. The walls were thickened and the

contents were of a semi-caseous nature. Microscopic sections showed clearly that the disease process had become firmly established. There was well marked congestion of all blood vessels throughout the organs. In some areas the capillary congestion had resulted in haemorrhage, although it is possible that these haemorrhages were the result of endothelial damage. Exudate under the mucous membrane was extensive and had caused rupture of the mucosa. This break in the continuity of the mucous membrane had allowed the escape of blood and inflammatory exudate into the caecal lumen (Fig. 6). There was widespread infiltration of all tissue layers by polymorphonuclear leucoytes and some lymphocytes (Fig. 7). Numerous protozoa with well defined eccentric nuclei and acidophilic cytoplasm were present; their appearance resembled the description of the vegetative phase of *Histomonas meleagridis* given by previous workers. In some areas the protozoal invasion had extended into the muscular layers. In the caecal contents vacuolated crescentic cells were present; these were probably oocyts or gametocytes of coccidia.

Livers. On this day all livers showed a number of pin-point lesions. In the liver sections there were numerous areas of well defined round cell infiltration. Frequently these foci were in close relation to blood capillaries and small bile ducts. There were some areas of necrosis. The cell nuclei were intact and the cell outlines were visible but the cytoplasm had disappeared. A few polymorphonuclear leucocytes were present but the cellular reaction was not marked. Protozoa were present in the round cell foci.

Day 6.

Caeca. In two poults the caecal changes were at the same stage as that described in day 5. In the third poult the lesions in the caeca were more advanced. The contents were caseous and solid, presenting a laminated structure on transverse section. On microscopic examination the histological reaction was relatively slight in material from two of the birds. Sections from the caeca of the third poult showed a marked polymorphonuclear inflammatory reaction accompanied by dilation of the capillaries. The leucocytosis was particularly marked in the submucosal areolar tissues and accompanied an extensive invasion by protozoa which extended to the muscle layers. Here protozoa could be seen lying between the individual muscle fibres (Fig. 8) and in the blood vessels. There was some evidence that the inflammatory reaction was beginning to involve the serosa.

Livers. The lesions were larger than at day 5, and were slightly raised above the surface of the organs. In sections the numerous areas of round cell infiltration were associated with necrosis of the hepatic parenchyma and the presence of protozoa. The distribution of this necrosis was irregular with ill defined

margins. In some cases the centre of the necrotic areas still contained isolated intact liver cells. The necrosis was karyolytic in type, the cell substance being "dissolved" out leaving only the cell outlines. There was an irregular distribution of some polymorphonuclear leucocytes, with no evidence of peripheral infiltration of the necrotic areas (Fig. 9). In one section there were early foreign body giant cells, some of which contained protozoa (Fig. 10). In another section there were a number of irregular, densely staining basophilic structures with little visible cytoplasm, which resembled the early invasive form of protozoa described by other workers.

Day 7.

On this day poults were showing clinical evidence of disease.

Caeca. These were in an advanced stage of inflammation with evidence of peritonitis on the serosal surface, extending to the adjacent mesentery and peritoneum. Microscopically a number of the intestinal villi were distended with masses of encysted protozoa (Fig. 11). Numerous pockets of protozoa had almost obliterated the host tissues. Polymorphonuclear leucocytes were very numerous. Protozoa had infiltrated through the muscle layers and pockets of fibrinous exudate containing occasional protozoa were seen lying on the serosal surface of the caeca.

Livers. These organs showed numerous large lesions accompanied by widespread congestion. Sections showed a variation in the age and size of the lesions. Many were small and showed only slight evidence of necrosis. Some of these lesions had developed from what had been foci of round cells near blood vessels. In older lesions necrosis was more prominent and polymorphonuclear leucocytes were common. Well stained protozoa were not frequently seen.

Day 8.

The poults were showing definite clinical symptoms.

Caeca. In one poult these were grossly enlarged. The walls contained numerous necrotic lesions and the contents of the lumen were solid. The caeca from the other two birds were in a less advanced stage of inflammation. Histologically it was evident that the process of inflammation was involving all the tissue layers of the organ, and there was an active peritonitis. In one section the mucous membrane had been destroyed and the underlying tissues were merging into the inflammatory debris in the caecal lumen. Protozoa could be seen in all parts of the sections, many of them lying together in so-called "nests" (Fig. 12).

Livers. Numerous large lesions were observed which closely resembled those seen in the natural disease. Microscopic examination revealed extensive areas of necrosis. These areas showed only the ghost outlines of the liver cells, interspersed with occasional

giant cells often containing protozoa (Fig. 13), and islets of apparently normal liver tissue. The whole process was characterised by a lack of polymorphonuclear cells which were so abundant in the caeca. However, in some places there were short fringes of round cells on the edge of the necrotic areas. Faintly staining protozoa, either encysted or dead, were common, apparently lying in the lacunae of destroyed liver cells.

Day 9.

Caeca. All caeca showed relatively advanced lesions although there was considerable variation in the degree of change between different caeca. In sections, the affected portions of the caeca presented an advanced peritonitis with fibrin, inflammatory polymorphonuclear cells and lymphocytes. In places the inflammatory process and protozoa had completely disrupted the normal arrangement of the muscle fibres in the muscular layer.

Livers. Again there was variation in the extent of the lesions and their size, which ranged from pin-head foci to large well defined lesions. Histological examination of one section presented the extensive area of necrosis described on previous days. In two other sections the necrotic areas were rich in protozoa with well defined cytoplasm. The lesions in these sections gave the impression of being at an early stage of development. In one or two places "nests" of such protoza lay in clear areas of lysed liver tissue and inflammatory and necrotic tissue changes were completely absent in the surrounding healthy tissue.

Day 10.

Caeca. Apart from the gross changes previously described there was an extending peritonitis which was involving the mesentery.

In one section where the mucous membrane had ruptured releasing dead tissue and inflammatory cells into the lumen, there was evidence of a fibroblastic reaction at the base of the lesion.

Livers. There was no further obvious development of the lesions. In the sections the protozoa were abundant. In some foci "nests" of protozoa were lying in close apposition to blood vessel walls. Some protozoa had penetrated the endothelium and they could be seen in the blood vessels (Fig. 14). Alternatively these protozoa may have been fresh arrivals from the caeca.

Day 11.

One of the poults due to be killed on this day was found dead, and another was moribund. Two other sick poults were also killed.

Caeca. All caeca showed advanced lesions with peritonitis involving the mesentery. The sections showed no new features, although in one section there was some fibroblastic activity near the muscularis mucosae and the mucous membrane was largely intact.

Livers. All livers contained widespread lesions, which microscopically were composed of sheets of necrotic tissue with apparently viable protozoa in the interstices.

Day 12.

Caeca. The caeca from the two surviving poults showed less severe changes than had been observed previously. On microscopic examination these findings were confirmed. One section appeared normal, the other only showed slight evidence of protozoal invasion.

Livers. The lesions in the livers showed little change from the preceding day. Histologically, in one liver the lesions were not large, although in the other there was extensive invasion by protozoa. In both cases the protozoa, which were acidophilic, stained well, and gave the appearance of being viable when fixed. In many cases they were arranged in tight mulberry-like clusters indicative of active multiplication. The general impression was that of early active lesions.

Spleen. In one spleen a small focus of protozoa was seen, with little surrounding tissue reaction or necrosis. Protozoa were also numerous in one blood vessel.

DISCUSSION

Interpretation of the foregoing results was complicated by the fact that two separate processes were taking place. On the one hand the invading protozoan was present in a variety of phases and as a result of this the defence mechanisms of the host tissues were stimulated, giving rise to a series of well-defined histopathological processes. Phases in the development of the protozoan have already been described by Tyzzer (1919) but unfortunately most of his illustrations are in the form of sketches and we have been unable to interpret, with confidence, many of these phases in our own material. We reproduce unretouched photomicrographs, which depict with reasonable accuracy the structures we have observed in sections of material taken from the experimental disease.

Our examinations of the caecal contents have confirmed the presence of the pear-shaped cells seen by Smith (1895) and Hadley and Amison (1911). On the other hand Smith (1915) and Tyzzer (1919) have both observed that the caecal contents abound with several species of protozoa, and the latter author has pointed out that in cases of blackhead the caecal contents frequently do not contain Histomonas. Apart from these pear-shaped cells which were also seen adhering to the caecal epithelium, all other protozoa observed have presented a more or less uniform appearance and we have been unable to distinguish clearly between vegetative and resistant phases.

The pathology of the disease follows a fairly well marked course. In the caeca there is an increase of protozoa in the con-

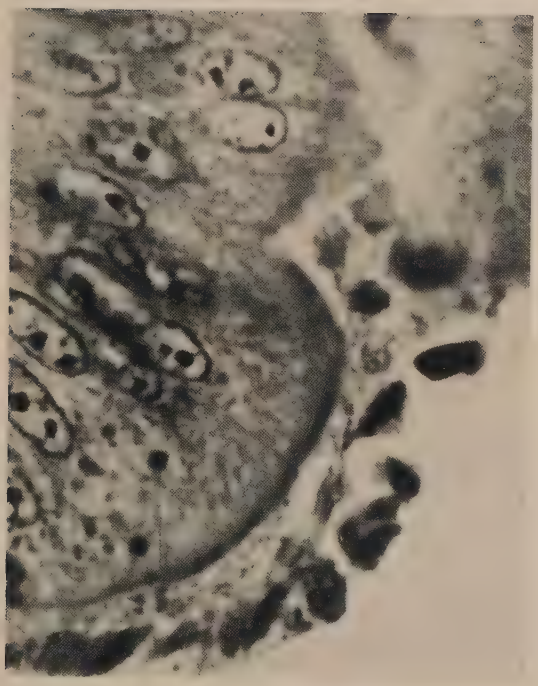
tents of the lumen during the first 24 hours and this is followed by hyperaemia and a polymorphonuclear leucocyte infiltration of the mucosa and submucosa. This infiltration is accompanied by an increase in lymphadenoid tissue. By the third day there is an extensive inflammatory reaction in the sub-mucosa, and at this time there is early histological evidence that changes are commencing in the liver. In view of the presence of parasites in caecal blood vessels, and later in hepatic vessels and even in the spleen, it seems clear that the protozoa pass to the liver by means of the blood stream.

Further development of the disease in the caecum is characterised by the appearance of cells with well defined eccentric nuclei, which were probably protozoa ingested by phagocytic cells. At this stage the inflammatory reaction is intense and accompanies protozoal infiltration which penetrates the muscular layers, eventually reaching the peritoneal surface. Peritonitis, with protozoa lying free in the cellular exudate is not uncommon, and this peritonitis often spreads by direct extension to the mesentery. We have not seen giant cells in caecal lesions at any stage of the disease, although these cells were described in that organ by Smith (1895) and Tyzzer (1919). It is probable that their material was from natural cases of a chronic nature. The disease in the caeca produced by our methods is an acute inflammatory process. However, in the later stages, i.e., on day 12, the lesions appeared to be less advanced. This may have been due partly to unavoidable variations in selection of material for section, and partly to varying degrees of natural resistance in the experimental birds. Alternatively, the process may have started to regress, since on the two previous days there was some evidence of fibroblastic activity in the submucous tissue. Further work on this and later stages in the disease is necessary to clear up this point.

Our studies have failed to provide conclusive evidence as to the mode of invasion in the caecum. It seems unlikely that Hadley's (1916) hypothesis of invasion via the goblet cells in the crypts of Lieberkühn provides the only mode of infection, as invasion of goblet cells has been observed in portions of the caeca other than the crypts, a view which has also been advanced by Tyzzer (1919). Furthermore no evidence has been forthcoming to support the views of Jowett (1911) who postulated that infection by histomonads followed a primary invasion by helminths and coccidia. Our methods of husbandry and housing of the poults would keep helminth burdens very low and in fact no helminths were ever seen in our post-mortem examinations.

The lesions in the liver appear to arise in areas adjacent to blood vessels. Initially these lesions are characterised by dense areas of round cell infiltration followed by cloudy swelling, degeneration and necrosis of the liver cells. At this stage protozoa are often seen associated with and ingested by giant cells.

Fig. 1



Day 1 : Caecum
H. & E. × 1,000
Columnar epithelium of villus tip showing adherent protozoa in mucoid contents.

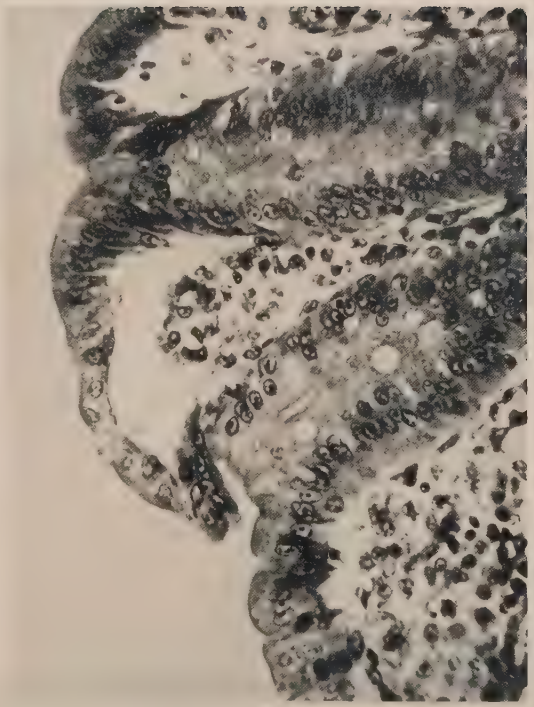


FIG. 2
Day 2 : Caecum
H. & E. × 700
Oedema of villus tips resulting in stripping of the epithelial layer.

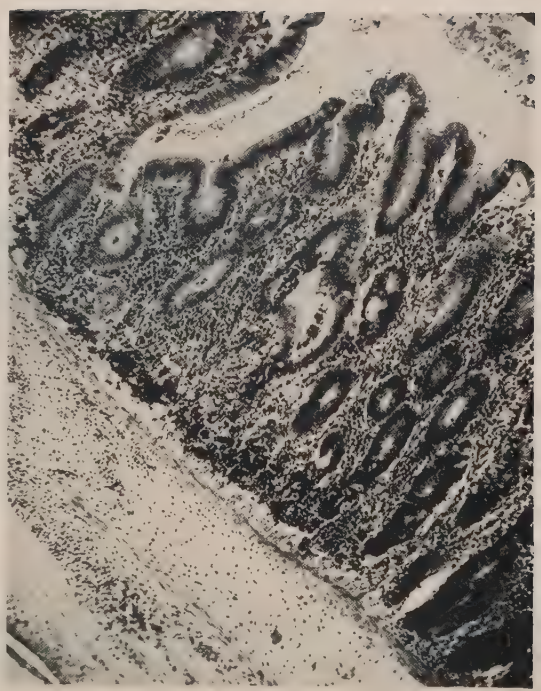


FIG. 3
Day 3 : Caecum H. & E. × 200
Oedema and generalised inflammatory reaction extending to the circular muscle layers.

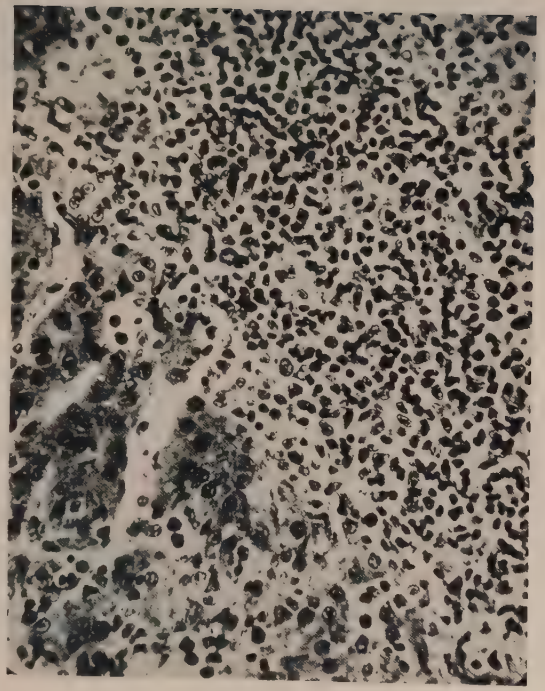


FIG. 5
Day 3 : Liver
H. & E. × 700
Portion of a focus of small round cells (early lesion).

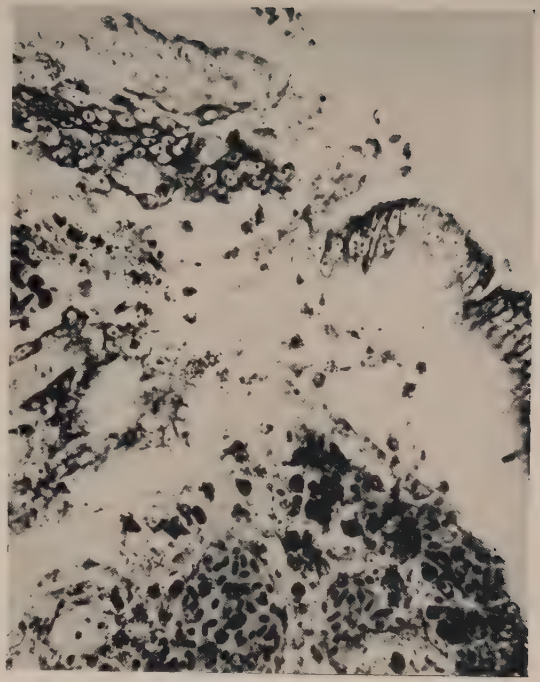


FIG. 6
Day 5 : Caecum
Giemsa × 700
Extensive oedema and inflammatory reaction resulting in desquamation.

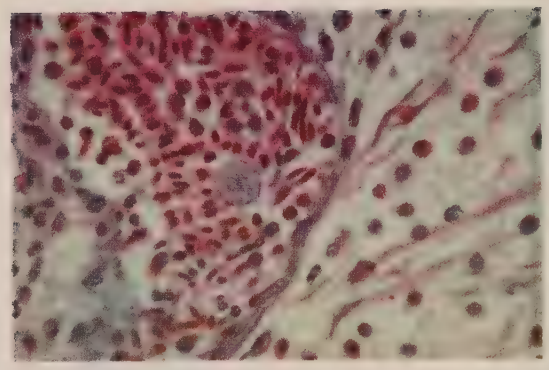


Fig. 4

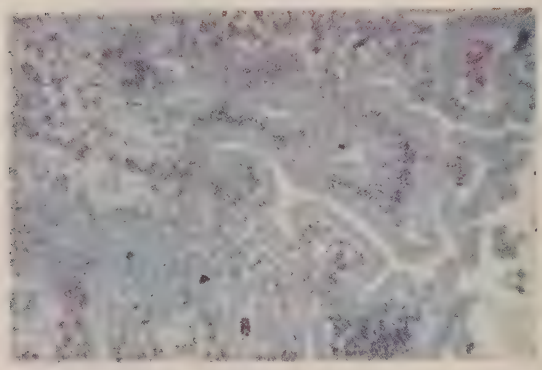


Fig. 7

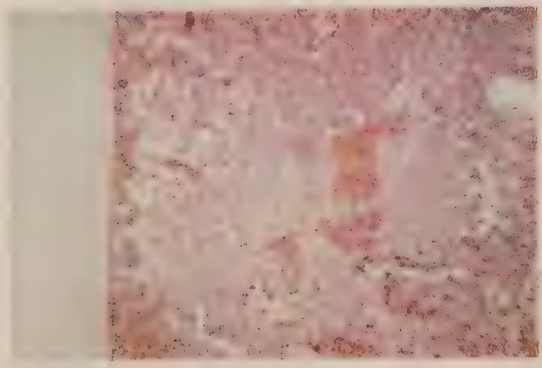


Fig. 9

Fig. 4
Day 3 : Caecum
H. & E. × 1.750
Small vein containing a histomonad.

Fig. 7
Day 5 : Caecum
Giemsa × 150
Lesion showing extensive infiltration by protozoa and accompanying inflammatory reaction, resulting in destruction of the normal architecture.

Fig. 9
Day 6 : Liver
H. & E. × 150
Established surface lesion showing necrosis of the parenchyma, haemorrhage, and some giant cells.

FIG. 8
Day 6 : Caecum
H. & E. × 700
Infiltration of the circular muscle bundle by protozoa. Note typical protozoal "nests."

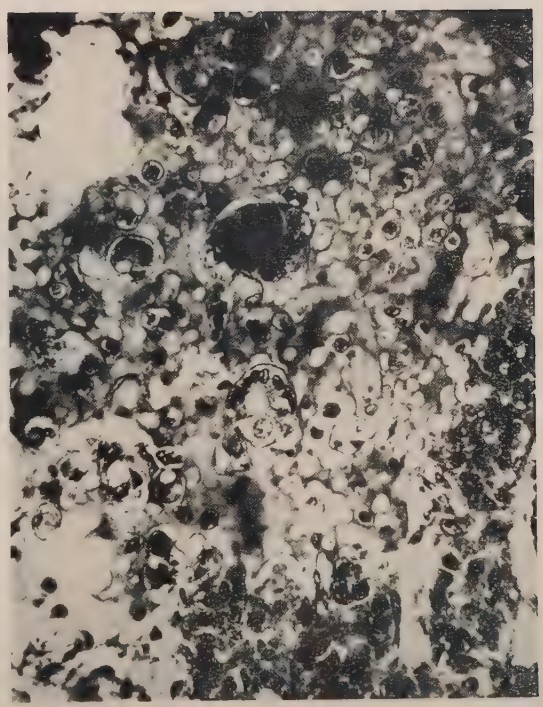


FIG. 10
Day 6 : Liver
H. & E. × 700
Portion of lesion similar to Fig. 9, showing giant cells in detail, some with ingested protozoa.

FIG. 11
Day 7 : Caecum H. & E. × 700
Portion of villus showing columns of " encysted " protozoa, and accompanying inflammatory cells.

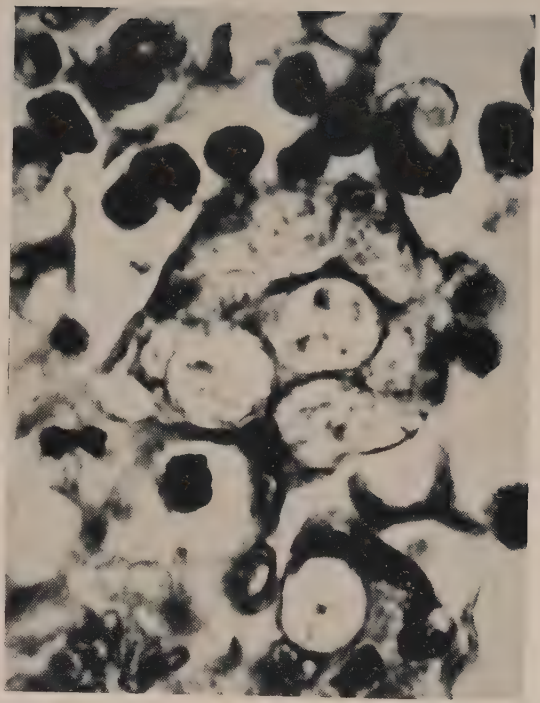


FIG. 12
Day 8 : Caecum
H. & E. × 1,000
" Nests " of protozoa in loose inflammatory connective tissues of the submucosa.

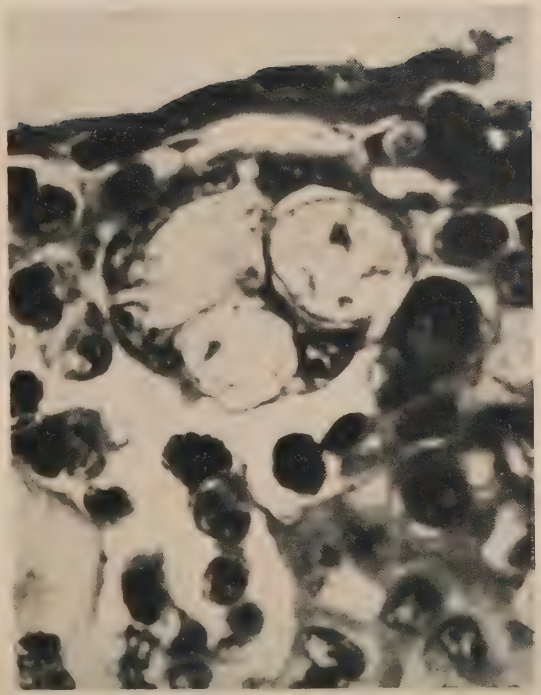


FIG. 13
Day 8 : Liver H. & E. × 1,000
Giant cell containing 3 protozoa, and surrounded by inflammatory cells at the edge of an advanced lesion.

FIG. 14
Day 10 : Liver
Giemsa × 1,000
Histomonads in a blood vessel.

As the disease progresses the multiplying protozoa can be seen as aggregations, resembling so-called "nests". At no stage after the early foci of round cells can the lesions be described as rich in inflammatory cells. There is apparently no attempt to wall off or invade the lesion from its periphery. The areas of necrosis extend and the lesions present a typical karyolytic appearance, with only the outlines of the liver cells and reticulum visible. The protozoa are seen lying in this reticulum. It was tempting to suppose that the destruction of the liver cells resulted from the presence of the protozoa within them. However, we were unable to find any evidence of protozoa in intact liver cells and it must be assumed that the protozoa migrate into these cell spaces after the death of the cells. This was also the opinion of Smith and Tyzzer. We have been unable to confirm Tyzzer's description of the presence of giant cells containing protozoa in the bile ducts, although they are common enough in the areas of liver necrosis.

The liver lesion in our experimental disease is essentially a karyolytic necrosis accompanied by a subacute type of inflammatory response. Our finding of protozoa in the spleen appears to be uncommon, although Levine (1947) has described Histomoniasis of the kidney in a turkey.

It was our intention to present a detailed account of the general histopathological changes which take place in the various organs involved in the disease. This object has been only partially fulfilled since the techniques used have failed to reveal information on the mode of invasion in the caecum, and the various phases in the life-cycle of the protozoan described by Tyzzer. A further investigation should be conducted with particular reference to the days immediately following infection. Serial sections of suitable material might provide more information on the pathogenesis of the disease, and more specialised techniques of fixation and staining would assist in the differentiation of the protozoal phases.

SUMMARY

A study has been made of the general histopathology of experimental infectious enterohepatitis (blackhead) in young turkey poults. The experimental infection was produced by the rectal injection of diseased caecal tissue from freshly killed infected turkeys. The material for study was collected from the experimental birds by killing 3 birds on each consecutive day after infection up to the 12th day.

It has been demonstrated that the infection spreads from the caeca to the liver via the blood stream, and that in the latter stages of the disease the spleen may become involved.

It has not been possible either to define the actual route of infection in the caeca or to add further information to the existing knowledge of the life cycle of the causative protozoon as it occurs in the host tissues.

ACKNOWLEDGMENTS

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LAPINISED RINDERPEST VIRUS AND A VACCINE: SOME OBSERVATIONS IN EAST AFRICA

I. Laboratory Experiments

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INTRODUCTION

Early observations on the adaptation of rinderpest virus to the rabbit have been recorded by Edwards (1924, 1927), Jacotot (1932), and Nakamura and his Japanese colleagues (1938, 1939). Haddow and Idnani (1947) experimented with a vaccine produced from rabbits but found that the virus lost invasive power for cattle after continuous serial passage in rabbits. However, Cheng and Fischman (1948) after a review of existing knowledge, described the successful use of lapinised rinderpest virus vaccine in China. Edwards (1949) recorded further experiments on the passage of the natural and a caprinised virus in rabbits.

The present work has been somewhat curtailed by routine duties; for example, the number of animals allotted to experiments was limited by the stabling available and the capacity of the vaccine section of the Institute to absorb control cattle challenged with bovine virus in order to utilise spleen for production of formolised-tissue vaccine. In addition, control animals challenged with virulent bovine rinderpest virus have had, on occasions, to be killed at the height of the temperature reaction before clinical symptoms developed. However, the challenge virus used is a standard product of the laboratory and it is not thought that any serious criticism can be made on that account. Routine checks were made in every case to rule out extraneous infections which might account for a temperature reaction. Some of the experiments incorporated here have been recorded in the Annual Report of the Kenya Veterinary Department, for 1949 (Kenya, 1951).

In the present paper the work of Cheng and Fischman (1948) is confirmed and experimental work with lapinised virus at this laboratory is recorded. Observations on the field use of vaccine prepared from rabbit tissue will be published later.

TECHNIQUE

Cattle. Susceptible steers from $2\frac{1}{2}$ to 5 years old and of known history were used. They were high- or medium-grade crosses of European breeds with native Zebu and all were kept in a single herd from which animals were drawn as required.

Rabbits. Rabbits were of the Albino and Rex breeds. They were laboratory-bred from stock maintained in excellent health and used when of 1000 g. weight at an age of over $4\frac{1}{2}$ months. Normal temperatures recorded showed an average of 101·6° F. in the morning and 102·4° F. in the evening.

Challenge Virus. The stock Kabete "O" strain was used. This has been maintained at Kabete by bovine passage for 30 years (Daubney, 1948) and virus is now prepared from spleen and stored after being freeze-dried; serial passage now takes place at intervals. The virus is of high virulence when injected subcutaneously, whether administered as fresh spleen or after freeze-drying.

Lapinised virus. We are indebted to Dr. K. L. Kesteven of the Food and Agricultural Organisation who sent dried virus by air from Nanking, China, in August 1948. This was the Nakamura Strain III which had been serially passaged in rabbits for 795 generations. Reactions in rabbits at Kabete were similar to those obtained in China, temperature rises being recorded 36 hours after infection. Kabete passages are indicated in the text by the letter "K" followed by a number; hence to the Kabete figure must be added 795 to give the total generations in the rabbit.

General. Virus was used either fresh or freeze-dried; the latter was prepared from dried blood, spleen and mesenteric lymph gland, suspended in normal saline and centrifuged at 2,000 r.p.m. for 10 minutes. Rabbits were injected intravenously, either with 1 ml. of fresh blood from an infected rabbit, or with 1 ml. of 1 : 10 dilution of dried material prepared as above. Temperatures were taken morning and evening for 64 hours and two-hourly between the 64th and 72nd hours, at which period the rise had reached its maximum and had begun to fall. Rabbits were killed as soon as this occurred, usually around the 70th hour.

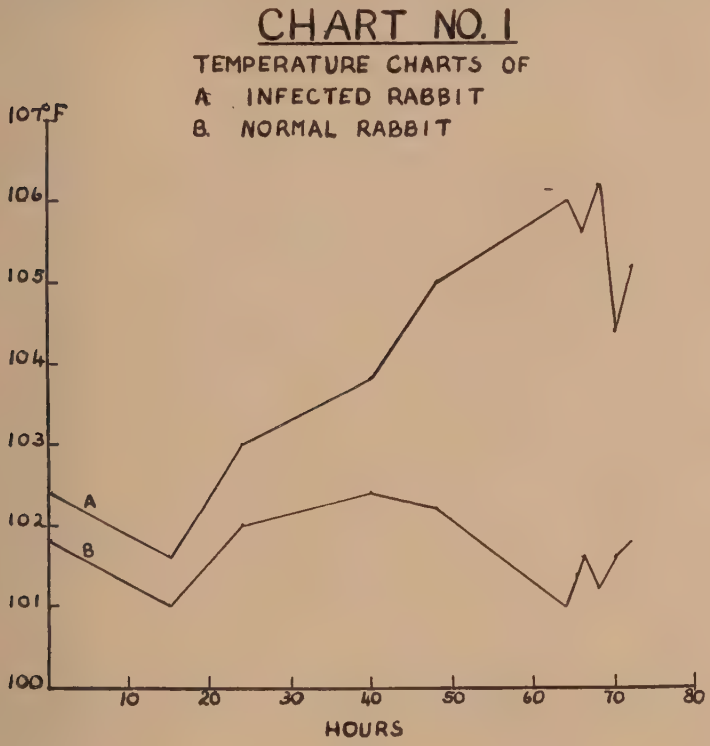
It was desirable that a dried product which could be standardised and easily stored should be used for the work since such a technique would ensure that the number of rabbit-passages was reduced to the minimum. A standard technique for the preparation of "seed" virus and vaccine has now been devised. Only blood, spleen and mesenteric glands are used, the blood serving as a diluent (Cheng and Fischman, 1948). All apparatus is sterilised and cooled beforehand. The organs are weighed, five times the weight of blood is added and the mixture is emulsified in a Waring Blendor. The emulsion is strained through gauze or glass wool and 1·5 ml. is placed in glass ampoules. The material is freeze-dried in an Edwards' Machine No. 3.P.S, drying being completed over phosphorus pentoxide on the secondary system of the machine. The ampoules of material which are sealed *in-vacuo* on the machine, have a residual moisture content of between one and two per cent. and are then stored at a temperature between minus 20 and minus 30° C.

THE VIRUS IN THE RABBIT

Symptoms.

After inoculation, rabbits appeared normal until the temperature began to rise, usually at the 36th hour after injection. During the period of fever, they showed discomfort until at the height of

the reaction the respirations were increased to the point of distress. Diarrhoea as described by Baker (1946) was not seen. A typical temperature reaction is shown in Chart I.



Thermal Reaction.

The average temperatures of 292 rabbits, of which 113 were infected by inoculation of fresh infected blood and 179 by inoculation of dried virus, showed little difference. Fresh blood produced a quicker response, but dried virus material induced a reaction to the same level which was sustained beyond the 64th hour as is shown by the following data: —

AVERAGE TEMPERATURES (°F.)

Hour	1	16	24	40	48	64	72
Fresh Blood	102·4	102·4	103·7	104·3	104·6	104·1	103·5
Dried Virus	102·4	102·4	102·6	103·6	104·6	104·4	104·4

Some rabbits failed to show a significant thermal reaction although typical lesions were present. This occurred only in young rabbits under $4\frac{1}{2}$ months of age, and in pregnant does.

In order to compare the reaction in a large number of rabbits, it was decided to regard 104° F. as the minimum temperature which constituted a thermal response; animals with a temperature below this but which showed typical lesions were considered to be "refractory". The syndromes in rabbits inoculated with fresh infected blood and those given dried virus were found to be similar, as shown in Table 1.

TABLE I

SYNDROMES: OBSERVATIONS ON 363 RABBITS.

Syndrome.	*Fresh Material.*	*Dried Material.*
Killed within 72 hours P.I. with temperature of over 104° F and with typical lesions	113 out of 150	179 out of 213
Died within 72 hours with typical lesions*	11 out of 150	7 out of 213
"Refractory" temperature but with typical lesions	26 out of 150	27 out of 213

* Distribution of Deaths P.I.

Hour.	*1*	*16*	*24*	*40*	*48*	*64*	*72*	*Total.*
Fresh Material ...	0	0	0	0	4	4	3	11
Dried Material ...	0	0	0	1	1	1	4	7

Inoculation by Other Routes.

Rabbits were successfully infected by intra-peritoneal injection. Infection was also established by the subcutaneous route, but the thermal reaction was delayed until the 48th hour and lesions were not well developed until after the 72nd.

Distribution of the Virus.

Dried blood, spleen and mesenteric gland each had a sufficient concentration of virus to immunise cattle in doses of 0·25 g. (Table II). Further details of this experiment are given later. As these were the proposed constituents of a vaccine, other possible sources were not investigated. Virus has been shown to be present in the urine of rabbits by C. T. Cheng *et al.* (quoted by Cheng and Fischman, 1948) and in the prescapular and precrural lymph glands and the urine of rabbits by Noble (1949).

Gross Lesions

Haemorrhagic streaks on the serous surface of the stomach and large colon may be present (23 out of292), while inflammation of the mucous membrane of the stomach and intestines has occurred

TABLE II

FIRST TEST OF DRIED LAPINISED MATERIAL IN CATTLE.

Cattle were injected with lapinised material on 20th November, 1948, and challenged on 2nd December, 1948, 13 days later.

Material Used.	*Cattle No.*	*Reaction to lapinised virus.*	*Reaction to challenge virus.*	*Result.*
0·25 g. K.6 Dried gland	6044	Rise to 104° F day 7 P.I. ...	Nil	Immune.
0·25 g. K.6 Dried spleen	6042	Nil	Nil	Immune.
0·25 g. K.6 Dried blood	6051	Nil	Nil	Immune.
———	6049	———	Virus control. Reacted	Killed for spleen vaccine.
———	6053	———	Virus control. Reacted	
———	6054	———	Virus control. Reacted	

infrequently (6 out of 292). The spleen was constantly enlarged and engorged. Baker (1946) also noted similar changes. Lesions were most marked in the lymphoid tissues of the intestine and the abdominal lymph glands. Peyer's patches, the appendix, the sacculus rotundus, the tonsilla caecalis major and the intestinal lymph glands, were swollen, greyish-white in colour and presented a nodular appearance similar to "elastoplast" or other adhesive tape. The mesenteric lymph gland in particular was very friable.

A common feature, not recorded in the literature, was a serogelatinous exudation around the mesenteric lymph gland. Cheng and Fischman (1948) quoting Nakamura and Fukusho (1939), reported that these lesions in the lymphoid tissue were also characteristic in rabbits infected with *Salmonella typhi-murium*. Attempts made at Kabete to isolate bacteria from rabbits showing these lesions were unsuccessful.

Histological Changes.

Comparative examination of sections of normal and infected rabbit tissues has shown that pathological changes are easily determined in all the lymphoid tissues mentioned. Axillary, precrural, prescapular and popliteal lymph glands showed microscopical changes when the naked-eye lesions were not obvious. The changes in the early stages were degeneration with loss of staining of the nuclei of lymphatic cells, followed by cellular necrosis and proliferation of reticulo-endothelial cells; degeneration was established by the 48th hour after infection and necrosis was always seen by 64th hour. In the early stages individual lymph follicles when stained by the routine haematoxylin and eosin method and viewed under the 2/3rds lens were prominent as lightly or unstained areas surrounded by dense masses of lymphocytes. Plates I and II illustrate the appearances of normal and infected tissues.

Change in Virulence on Rabbit Passage.

No change in virulence for the rabbit has been noted following the 21 passages made at Kabete. There has been a delay of the thermal reaction until the 48th hour and a lowering of the peak temperature on some occasions when stored dried virus has been injected; but one or two direct blood passages have restored the syndrome. This modification of the reaction was ascribed to insufficient drying of the material before storage, with a consequent lowering of the virus content. No alteration of the macroscopic or microscopic lesions was observed.

Susceptibility in Relation to Breed, Age and Sex

No deliberate attempt was made to investigate this point, but during the experiments covering about 360 inoculations it became obvious that young light-weight rabbits were not good subjects. Those below $4\frac{1}{2}$ months of age were refractory as regards thermal

response and pregnant does also gave poor reactions. All breeds of rabbits used at this laboratory have proved susceptible. Black Rex breeds have a large quantity of mesenteric fat and this proved inconvenient when removing glands for vaccine production.

SURVIVAL OF THE VIRUS.

Survival of the virus in dried tissues. Cheng and Fischman (1948) recorded that dried vaccine packed *in-vacuo* maintained potency at 4° C. for 105 days. In this laboratory ampoules of dried material each containing 0·5 g. under vacuum, were placed on the bench out of direct sunlight; a thermograph recorded a maximum temperature of 24° C. and a minimum of 18° C. throughout the experiment. One ampoule was titrated in rabbits at intervals with the following results: —

Original titre	0·00001 g.
Titre after 10 days	0·0001 g.
„ „ 14 „	0·001 g.
„ „ 30 „	0·01 g.
„ „ 70 „	0·01 g.

Other experiments were carried out and material again survived at high titres for periods of over 7 days at this ambient temperature. Further experiments are in progress to test both in rabbits and in cattle viability at room temperature and at 37° C. of material in ampoules under vacuum and of reconstituted fluid.

Dried virus stored *in vacuo* in the refrigerator at between minus 20° C. and minus 30° C. has remained viable for 25 months. No difficulty has been experienced in sending vaccine to the field, provided that reasonable precautions were taken and the ampoules packed on ice.

Survival of the virus in glycerine. Cheng and Fischman (1948), quoting from the unpublished records of Ma and Fang, reported that the virus in fresh spleen and lymph gland survived for 17 to 20 days when stored in glycerine and buffered saline (1 : 1) at 5° C. Dried viable material suspended in this medium was not viable after 24 hours at Kabete. Quantities of the same virus material kept at atmospheric pressure and at the same temperature (18° C. to 24° C.) maintained an infective titre for rabbits of 0·001 g. for 7 days.

TRANSMISSION OF LAPINISED VIRUS IN CATTLE

Alternate Passages

Nakamura and Kuroda (1942) concluded that lapinised virus injected into calves and then passaged 10 times in calves exhibited no exaltation of virulence for these animals, while pathogenicity for the rabbit was not altered at the conclusion of the experiment. At Kabete, cattle tested with lapinised virus have shown a very mild reaction only; symptoms have been confined to a rise in temperature of two degrees Fahrenheit above the normal when material

was inoculated at a dilution of 1 : 10 and in a few only of the many cattle tested at this dilution. This lack of reaction made it impossible to determine whether cattle had been immunised until after a challenge had been made with virulent bovine virus. The alternate passages as shown in the following chart were therefore carried out in cattle and rabbits, with the object of exalting the virulence sufficiently to provoke a constant thermal reaction in cattle. (Chart II.)

CHART II.

ALTERNATE PASSAGES IN RABBITS AND CATTLE

	Passage Designation. RABBIT. Passage K. 11	*Result* RABBIT	PASSAGE
(i)	K. 11 × B.	4 CATTLE.	IMMUNE ON CHALLENGE.
(ii)	K. 11 × B × R.	Rabbits.	LESIONS MARKED.
(iii)	K. 11 × B × R × B.	4 CATTLE.	IMMUNE ON CHALLENGE.
(iv)	K. 11 × B × R × B × R.	Rabbits.	LESIONS WEAK.
(v)	K. 11 × B × R × B × R_2	Rabbits.	LESIONS MARKED.
(vi)	K. 11 × B × R × B × R_2 × B.	4 CATTLE.	2 IMMUNE ON CHALLENGE. 2 SUSCEPTIBLE ON CHALLENGE.
(vii)	K. 11 × B × R × B × R_2 × B × R.	Rabbits.	NO LESIONS.
(viii)	K. 11 × B × R × B × R_2 × B × R_2	Rabbits.	NO LESIONS.

Dried virus material was inoculated into cattle, in quantities of 0·1 g., 0·01 g., 0·001 g., and 0·005 g. and the 4 cattle were housed with an uninoculated control. On the appearance of a thermal reaction, the cattle were bled on two successive days, the blood pooled and inoculated intravenously into rabbits. These rabbits were killed at the usual time after injection (about 70th hour). The material obtained was freeze-dried as usual, and again inoculated into cattle in the quantities previously described. Cattle were observed for 14 days and then challenged. In this manner it was possible to observe the progress of the alternate passage: in cattle the thermal and immunising effects could be followed and in the rabbits the thermal reactions and the lesions which

denoted the presence of the virus could be seen. The following comments are necessary.

(i) Only two cattle (0·1 g. and 0·001 g.) out of four used showed slight thermal reactions on the 6th and 7th day after injection and were bled. During 14 days all the cattle remained otherwise normal and were then challenged; all four proved immune and the control reacted severely.

(ii) One ml. of pooled bovine blood passage K. 11 × B was inoculated into each of 4 rabbits; three showed a mild thermal reaction commencing at the 60th hour and when killed at 72nd hour all exhibited typical lesions of rinderpest as seen in the rabbit. Material was freeze-dried and stored.

(iii) Cattle were inoculated as before with quantities of passage K.11 × B × R and one (0·1 g.) showed a thermal reaction on the 5th, 6th and 7th days after injection. All cattle were bled on the 6th and 7th days. Again, the animals remained normal and were challenged on the 14th day; all proved immune except the uninoculated control.

(iv) Four rabbits received 1 ml. each of blood passage K.11 × B × R × B; one died at the 24th hour while three were killed at the 66th hour having shown no temperature reaction. Two showed poorly developed lesions and one typical lesions of rinderpest. An uninoculated control rabbit remained normal in temperature and showed no lesions on autopsy.

(v) As the previous passage in rabbits revealed poor reactions one further rabbit passage was carried out from the one typical case. Three rabits reacted showing a temperature rise from the 48th hour and typical lesions on autopsy. Material was dried and stored as K.11 × B × R × B × R_2.

(vi) Dried material K.11 × B × R × B × R_2 was inoculated into 4 cattle as before. Two (0·1 g. and 0·001 g.) reacted severely between the 8th and 12th day; both showed high temperatures, became dull and refused to eat while the buccal mucosa was dry and inflamed. Examination revealed no extraneous cause for these symptoms and on subsequent challenge these cattle proved immune while the other two together with the control, remained normal throughout and reacted severely to challenge. Reacting cattle (0·1 g. and 0·001 g.) were bled on the 7th and 8th days after injection.

(vii) Bovine blood failed to provoke a temperature or lesions in four rabits when 1 ml. of K.11 × B × R × B × R_2 × B was inoculated.

(viii) Material from these rabbits collected at the 72nd hour and inoculated into four more rabbits likewise failed to provoke a response.

In view of the severe reactions in the two cattle, which could only be attributed to the virus, the experiment had to be discon-

tinued, due to the danger of using in the Attenuated Virus Unit of the Institute a virus of apparently exalted virulence.

In section (vi) the fact that the cattle which received 0·01 g. and 0·005 g. failed to become protected after apparently receiving a much more active virus, while those which received 0·1 g. and 0·001 g. reacted severely with subsequent immunity, requires some explanation. It may be that the critical range of the titre of virus lay between 0·1 and 0·01 in the dilutions which were used. The virus is contained in the relatively coarse dried organs, and in this instance, while there was not sufficient virus equally distributed in the 0·01 dilution to cause a reaction, a quantity of virus attached to particulate matter may have been carried over to the 0·001 dilution and this was sufficient to provoke a reaction and subsequent immunity. A similar phenomenon frequently occurs when titrating goat-adapted virus near the end-point of the dilutions and the consequent "misses" are attributed to uneven distribution of the virus and a "carry-over" by minute particles of tissue. Passage material has been stored and the whole problem will be investigated when suitable accommodation becomes available.

It was concluded, however, that lapinised virus could be recovered from cattle and passaged in rabbits, but that after two alternate bovine-rabbit passages, the clinical syndrome in the rabbit was altered and the virulence for cattle was apparently exalted.

Transmission of Virus by Contact.

Uninoculated rabbits have been kept together with experimental rabbits but these have never shown temperature reactions or lesions of rinderpest on autopsy. Many such controls have been challenged with lapinised virus and have reacted typically. Uninoculated cattle were always kept in close contact with cattle inoculated with lapinised material and all such control animals have remained healthy and have reacted to challenge with bovine virus after 14 to 21 days of contact. No evidence has been found to indicate that the virus will pass from infected rabbits to susceptible rabbits, or from infected cattle to susceptible cattle.

TITRATION EXPERIMENTS IN CATTLE AND RABBITS

First Immunity Test in Cattle

Two rabbits were injected with virus K.5. Blood from both rabbits was pooled and dried, the spleens were emulsified in normal saline, pooled and dried and the mesenteric glands were similarly treated. Three cattle were removed from a group of 15. No. 6051 was given 0·25 g. of dried blood, reconstituted in 5 ml. normal saline; No. 6042 received 0·25 g. of dried spleen and No. 6044, 0·25 g. of dried mesenteric gland, all by subcutaneous injection. On the same day, the 12 remaining cattle were infected with virulent bovine virus. Five days later these 12 had all

reacted and were killed for production of spleen vaccine. Table II shows results in the three vaccinated cattle and three controls. It therefore seemed reasonable to conclude that 6051, 6042 and 6044 were also susceptible before inoculation with lapinised material. They were observed for 13 days and exhibited no symptoms, except that No. 6044 showed a temperature rise on the evening of the 7th day. On the 13th day they were challenged and showed no symptoms for a further 35 days, although during this period they were housed in the block of stables reserved for the infection of cattle with rinderpest virus. It was concluded that dried blood, spleen and gland from rabbits infected with lapinised rinderpest virus had retained a titre sufficient to immunise the cattle.

Titration of Fresh and Dried Virus-Material in Rabbits

Material from rabbits infected with passage K.9 was emulsified and 2 g. removed for titration; the remainder was freeze-dried and another 2 g. removed for titration. Intravenous injection of serial tenfold dilutions in normal saline of the fresh material showed that 1 g. contained 1,000,000 infective doses; unfortunately titration was not carried beyond this point, because such a high titre was not anticipated. (Table III.) Serial dilutions of the dried virus showed that 1 g. contained 100,000 infectve doses. (Table V.) The 50 per cent. end-point method of Reed and Muench (1938) would have been preferable but at this time there was a shortage of rabbits. In both these titration experiments, positive rabbit reactions were based on temperature reactions and on typical lesions on autopsy. In the case of fresh material, blood from an animal reacting to the 10^{-6} dilution was sub-inoculated into other rabbits, which reacted typically; two cattle were also inoculated with this blood. One ox died from extraneous causes, but the other proved immune while two controls reacted severely to the challenge virus. (Table IV.) Heart blood from reacting rabbits was examined bacteriologically and no organisms were demonstrated.

Titration of Virus in Rabbits and Cattle

Material from passage K.13 was prepared in the usual way and titrations carried out in rabbits and in cattle, before and after the material was freeze-dried.

Fresh material. Serial dilutions in rabbits indicated that 0·0000005 g. in 0·5 ml. of saline caused the typical syndrome when injected intravenously. (Table VI.) The minimum quantity of material which would immunise an ox was 0·001 g. when injected subcutaneously in 1 ml. of saline. (Table VII.)

Dry material. Serial dilutions in rabbits showed that 0·00001 g. was the least amount of material which would infect a rabbit. (Table VIII.) The least quantity which would immunise an ox was 0·001 g. (Table IX.)

TABLE III

TITRATION OF FRESH MATERIAL K.9 IN RABBITS.

No.	*Dose in grammes.*	*Temperature reaction.*	*Day Examined.*	*Lesions.*	*Virus Potency.*	*Remarks.*
R.1	0·000001	Acute	Retained	—	plus	Survived.
R.2	0·000001	Acute	day 4	typical	plus	Bled for sub-inoculation into G.1.
R.3	0·00001	Acute	day 4	typical	plus	Killed. Bacteriological examination negative.
R.4	0·00001	Acute	day 4	typical	plus	Died. Bacteriological examination negative.
R.5	0·0001	Acute	day 8	typical	plus	Killed. Bacteriological examination negative.
R.6	0·0001	Acute	day 8	typical	plus	Died. Bacteriological examination negative.
B.1	0·001	Acute	day 8	typical	plus	Died. Bacteriological examination negative.
B.2	0·01	Acute	day 10	———	plus	Died, advanced P.M. changes.
B.3	0·1	Acute	day 4	typical	plus	Died, septic metritis.
B.4	———	Normal	day 10	Nil	——	Killed, no lesions present.
G.1	1 ml. blood	Acute	Retained	———	plus	Subinoculated from R.2 above.
G.2	1 ml. blood	Acute	day 9	typical	plus	Subinoculated from R.2 above, bled day 4.
X	1 ml. blood	Acute	day 6	typical	plus	Subinoculated from G.2.

TABLE IV

CHECK IN CATTLE FOR THE PRESENCE OF VIRUS IN BLOOD OF RABBIT REACTING TO K.9 FRESH 10^{-6}

Cattle were injected with blood on 9th December, 1948, and challenged on 24th December, 1948, 12 days later.

Cattle No.	*Inoculations.*	*Reaction to Inoculation.*	*Reaction to Challenge.*	*Result.*
6124	10 ml. blood ex R.2 ...	Marked temperature.	Died E.C.F. before challenge...	
6125	10 ml. blood ex R.2	Nil	Nil	Immune.
6133	———	———	Virus control, reacted	Killed for spleen vaccine.
6134	———	———	Virus control, reacted	

TABLE V

TITRATION OF DRIED MATERIAL K.9 IN RABBITS.

No.	*Dose in grammes.*	*Temperature reaction.*	*Day examined.*	*Lesions.*	*Virus Potency.*	*Remarks.*
1.	0·000001	Nil	Day 15	Nil	——	
2.	0·000001	Nil	Day 15	Nil	——	
3.	0·00001	Acute	Retained	——	plus	Bled for subinoculation into 11 on day 4.
4.	0·00001	Acute	Day 14	Typical	plus	Died. Secondary infection of necrotic lesions.
5.	0·0001	Good	Retained	——	plus	Survived.
6.	0·0001	Acute	Day 11	Typical	plus	Died. Secondary infection of necrotic lesions.
7.	0·001	Acute	Day 7	Typical	plus	Killed. Bacteriological examination negative.
8.	0·01	Acute	Retained	——	plus	Survived.
9.	0·1	——	Day 2	Nil	——	Died day 2 from ruptured stomach.
10.	——	Normal	Day 15	Nil	——	Control. No lesions present.
11.	1 ml. blood	Acute	Day 6	Typical	plus	Killed. Bacteriological examination negative.

TABLE VI

TITRATION OF K.13 (FRESH) IN RABBITS.

No.	*Dose in grammes.*	*Temperature Reactions.*	*Day Examined*	*Lesions.*	*Virus Potency.*	*Remarks.*
B.4	0·0000005	Acute Delayed	Day 5	Present	Plus	Killed. Bacteriological examination, negative.
B.3	0·0000005	Nil	Day 5	Good	Plus	Killed. Lesions mild in character. Bled.
B.2	0·000001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
B.1	0·000001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
R.4	0·00001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
R.3	0·00001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
R.2	0·0001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
R.1	0·1	Acute	Day 5	Typical	Plus	Died. Bacteriological examination negative.
Control.	———	Normal	Day 8	Nil	——	No lesions present.

TABLE VII

TITRATION IN CATTLE OF K.13 (FRESH).

Cattle were injected with lapinised material on 14th January, 1949, and challenged on 27th January, 14 days later.

Cattle No.	*Dose in grammes.*	*Reaction to lapinised virus.*	*Reaction to Challenge.*	*Result.*
6404	0·00001	Nil	Reacted. Temperature and mild symptoms ...	Susceptible.
6403	0·00001	Nil	Reacted. Temperature and typical symptoms ...	Susceptible.
6402	0·0001	Nil	Reacted. Temperature and typical symptoms ...	Susceptible.
6400	0·0001	Nil	Slight evening temperature on day 5—7. ...	Partial immune.
6386	0·001	Nil	Nil	Immune.
6380	0·001	Nil	Nil	Immune.
6384	0·01	Nil	Nil	Immune.
6397	0·1	104° F. day—8 P.I.	Nil	Immune.
6406	Nil	———	Virus control. Reacted typically, survived ...	——
6390	Nil	———	Virus control. No reaction. Immune Ox ...	——
6383	Nil	———	Virus control. Reacted typically, survived ...	——
6382	Nil	———	Virus control. Reacted. Died rinderpest ...	——

TABLE VIII

TITRATION OF K.13 (DRIED) IN RABBITS.

No.	*Dose in grammes.*	*Temperature reaction.*	*Day Examined.*	*Lesions.*	*Virus Potency.*	*Remarks.*
8	0·0000005	Nil	Retained	——	Nil	Challenged day 8 with lapinised virus. Reacted.
7	0·0000005	Nil	Day 6	Nil	Nil	Killed.
6	0·000001	Nil	Retained	——	Nil	Challenged day 8 with lapinised virus. Reacted.
5	0·000001	Nil	Day 7	Nil	Nil	Killed.
4	0·00001	Acute	Day 5	Typical	Plus	Killed. Bled for subinoculation.
3	0·00001	Acute	Day 5	Typical	Plus	Killed. Bled for subinoculation.
2	0·0001	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
1	0·1	Acute	Day 5	Typical	Plus	Killed. Bacteriological examination negative.
Control.	Nil	Nil	Day 7	Nil	——	No lesions present.

TABLE IX

TITRATION IN CATTLE OF K.13 (DRIED).

Cattle were injected with lapinised material on 15th January, 1949, and challenged on 27th January—13 days later.

Cattle No.	*Doses in grammes.*	*Reaction to lapinised virus.*	*Reaction to Challenge.*	*Result.*
6396	0·00001	Nil	No symptoms. Immune animal?	——
6392	0·00001	Nil	Reacted. Typical symptoms of rinderpest. Died.	Susceptible.
6394	0·0001	Nil	Irregular evening temperatures. No symptoms ...	Partial Immune.
6393	0·0001	Nil	Temperature rise day 4 to 6. No other symptoms ...	Partial Immune.
6399	0·001	Nil	Nil	Immune.
6389	0·001	Nil	Slight temperature only day 2 and 3 P.I.... ...	Immune.
6387	0·01	Temperature days 7—9	Nil	Immune.
6391	0·1	Temperature days 6—8	Nil	Immune.
6395	Nil	———	Virus control. Reacted typically. Survived ...	———
6398	Nil	———	Virus control. Reacted typically. Died	———
6391	Nil	———	Virus control. Reacted typically. Survived ...	———
6395	Nil	———	Virus control. Reacted typically. Survived ...	———

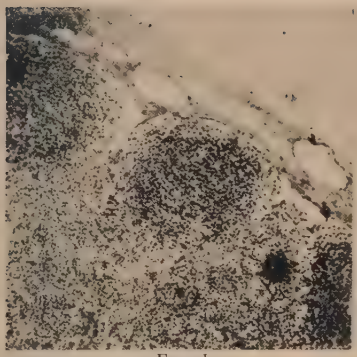


FIG. 1

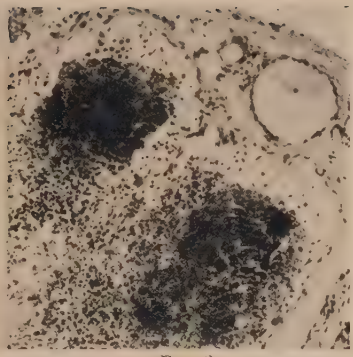


FIG. 2

FIG. 1. Mesenteric Lymph Gland. Normal appearance of nodes. H.E. × 150.

FIG. 2. Mesenteric Lymph Gland showing necrotic areas in the nodes. H.E. × 150.

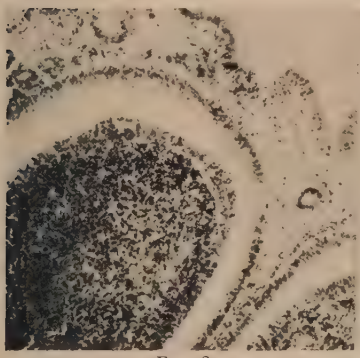


FIG. 3

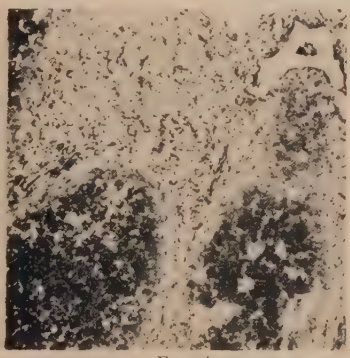


FIG. 4

FIG. 3. Appendix from uninfected rabbit. H.E. × 150.

FIG. 4. Appendix of rabbit killed at hour 72 p.i., showing necrosis of lymphoid tissue. H.E. × 150.

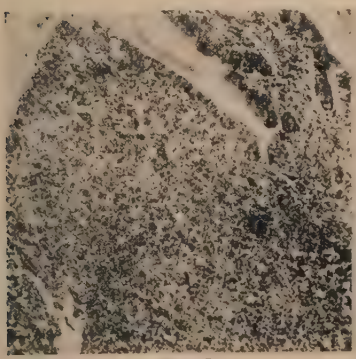


FIG. 5

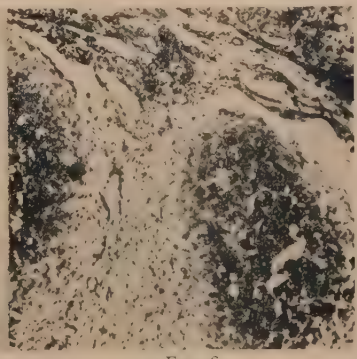
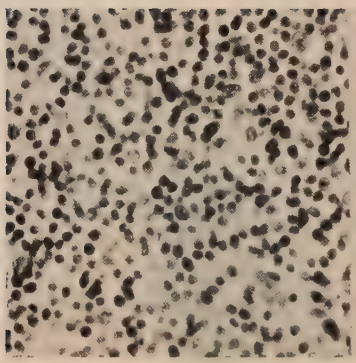


FIG. 6

FIG. 5. Payer's patch from uninfected rabbit. H.E. × 150.

FIG. 6. Payer's patch of rabbit killed at hour 72 p.i. showing extensive necrosis. H.E. × 150.



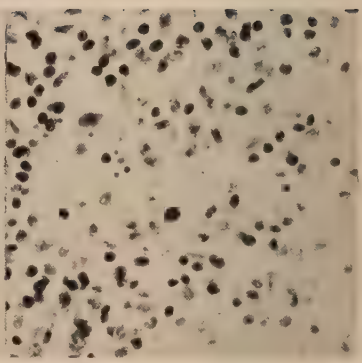
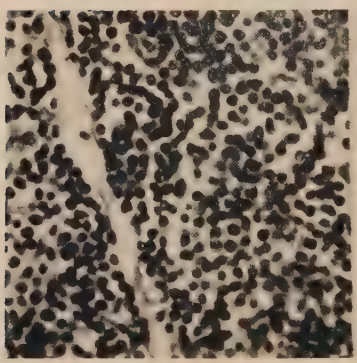


Fig. 7 Fig. 8

Fig. 7. Area from a normal Axillary Lymph Gland. H.E. × 650.

Fig. 8. Area from Axillary Gland of rabbit killed at hour 72 p.i., showing degeneration of lymphocytes and infiltration of reticulo-endothelial cells. H.E. × 650.



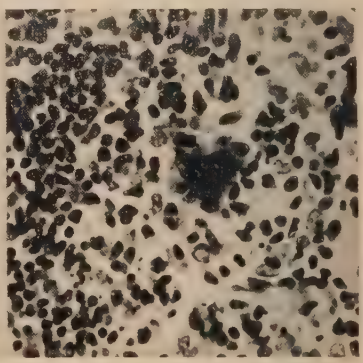


Fig. 9 Fig. 10

Fig. 9. Area from a normal Popliteal Lymph Gland. H.E. × 650.

Fig. 10. Area from Popliteal Gland of rabbit killed at hour 72 p.i., showing the demarcation of normal and affected cells. H.E. × 650.

TABLE X

CHECK IN CATTLE FOR THE PRESENCE OF VIRUS IN BLOOD OF RABBITS REACTING TO K.13, FRESH AND DRIED.

Cattle were injected with blood on 18th and 19th January, 1949 and challenged on 27th January, ten days later.

Cattle No.	*Inoculation.*	*Reaction to inoculation.*	*Reaction to challenge.*	*Result.*
6413	5 ml. blood from rabbit B 4... ...	Temperature day 5 ...	Nil	Immune.
6414	5 ml. blood from rabbits 3 and 4 ...	Temperature days 4 and 5	Nil	Immune.
6415	———	———	Virus control, died rinderpest.	——

Rabbits reacting to the highest dilutions in each group were bled and cattle were injected subcutaneously with 5 ml. of this blood. When challenged 14 days later these cattle proved immune while an uninoculated control succumbed to the virulent virus, thus proving that the rabbits were reacting to lapinised rinderpest virus. (Table X.)

It was concluded that drying of the virus material had little effect upon the titre which conferred immunity to cattle. It was not practical, however, on the evidence of one experiment, to relate with certainty the minimum quantity which would infect a rabbit to the minimum quantity which would immunise on ox; in the former case the criterion of infection was a visible disease syndrome while in the latter case, immunity, in the absence of any reaction to the virus, could only be estimated by challenge. It was essential, therefore, before lapinised virus was used in the field that the potency of each batch of vaccine should be tested by titration of the vaccine in cattle.

EXPERIMENTS WITH A VACCINE IN CATTLE

Titration of Vaccine in Cattle

Having once established the fact that 0·001 g. of dried material was sufficient to protect an ox, experiments were continued with a view to producing a standardised vaccine. Many cattle were tested with material made by the method described under "Technique" and some examples of tests in cattle with this "lapinised" vaccine are shown in Tables XI and XII. By improving technique and by using more efficient methods of freeze-drying, the minimum amount of dried material which would immunise an ox was reduced from 0·001 g. to 0·0001 g. In all titrations an uninoculated animal was included as a control. It was concluded that the vaccine would regularly protect grade and low-grade cattle under laboratory conditions of test.

Exposure of Vaccinated Cattle to Contact Infection

Cattle injected with lapinised virus vaccine have been exposed to contact with cattle reacting to the "O" strain of Kabete bovine virus and have failed to become infected with rinderpest. It seemed probable that exposure to the Kabete "O" virus was not a sufficiently severe challenge, so the experiment was later repeated. Other cattle injected with lapinised vaccine were exposed to contact with a recently-isolated field strain of virulent rinderpest and the experimental protocols will be described in a latter paper.

Test of the "Interference Phenomenon"

Pfaff (1938) showed that some protection was afforded to cattle 24 hours after injection with goat-adapted rinderpest virus and Daubney and Hudson (1939) showed that this protection was absolute at 72 hours. This was considered to be the result of "interference" by the attenuated virus preventing the multiplica-

TABLE XI

SOME EARLY TESTS IN CATTLE OF DRIED LAPINISED VIRUS VACCINE.

Virus.	*Dose in grammes.*	*Cattle No.*	*Date of injection.*	*Reaction to Vaccine.*	*Interval between test and challenge.*	*Reaction to Challenge.*	*Result*
K.19	0·004	7189	31.3.49	Nil	7 days	Slight temperature, no symptoms	Immune.
	0·004	7188	31.3.49	Nil	7 days	Nil	Immune.
	0·004	7187	31.3.49	Temperature day 6—7 p.i.	7 days	Off-feed 5 days, no other symptoms	Immune.
	——	7256 to	——	——	——	Virus controls. All reacted, and	——
	——	7259	——	——	——	killed for spleen vaccine.	——
K.20	0·004	7714	23.4.49	Temperature day 6 p.i.	13 days	Reacted but recovered	Susceptible
K.20	0·004	7732	23.4.49	Nil	13 days	Nil	Immune.
K.21	0·004	7723	23.4.49	Nil	13 days	Nil	Immune.
K.21	0·004	7735	23.4.49	Nil	13 days	Nil	Immune.
K.22	0·004	7711	23.4.49	Nil	13 days	Nil	Immune.
K.22	0·004	7508	23.4.49	Temperature day 5 and 6 p.i.	13 days	Nil	Immune.
	——	7853 to	——	——	——	Virus controls, all reacted and	——
	——	7856	——	——	——	killed for vaccine.	——
K.16 C	0·004	8746	19.10.49	Temperature day 6 and 7	7 days	Nil	Immune.
K.16 C	0·004	8744	19.10.49	Temperature day 6 and 7	7 days	Temperature reaction day 3—6	Partial Immune.

TABLE XII

SOME TITRATIONS IN CATTLE OF LAPINISED VIRUS ISSUED AS VACCINE TO THE FIELD.

Virus.	*Dose in grammes.*	*Cattle No.*	*Date of injection.*	*Reaction to Vaccine.*	*Interval between test and challenge*	*Reaction to Challenge.*	*Result*
K.11	0·005	9424	22.4.50	Nil	14 days	Nil	Immune.
	0·001	9417	22.4.50	Nil	14 days	Nil	Immune.
	0·01	9425	22.4.50	Nil	14 days	Nil	Immune.
	0·1	9418	22.4.50	Temperature reaction days 5—9 p.i.	14 days	Nil	Immune.
	Control	9416	——	In contact control. Nil	14 days	Reacted, killed vaccine	——
K.17	0·005	122	8.7.50	Nil	13 days	Nil	Immune.
	0·001	124	8.7.50	Nil	13 days	Nil	Immune.
	0·01	129	8.7.50	Nil	13 days	Nil	Immune.
	0·1	126	8.7.50	Temperature reaction days 6—9 p.i.	13 days	Nil	Immune.
	Control	123	——	In contact control. Nil	13 days	Reacted, killed vaccine	——
K.18	0·0001	769	11.9.50	Nil	15 days	Nil	Immune.
	0·00025	765	11.9.50	Slight temperature days 7—9 p.i.	15 days	Nil	Immune.
	0·0005	767	11.9.50	Slight temperature	15 days	Nil	Immune.
	0·01	771	11.9.50	Nil	15 days	Nil	Immune.
	Control	764	——	In contact control. Nil	15 days	Reacted, killed vaccine	——
K.20	0·0001	1127	15.1.51	Nil	11 days	Nil	Immune.
	0·00025	1130	15.1.51	Nil	11 days	Nil	Immune.
	0·0005	1120	15.1.51	Nil	11 days	Nil	Immune.
	0·1	1125	15.1.51	Nil	11 days	Nil	Immune.
	Control	1122	——	In contact control. Nil	11 days	Reacted, killed vaccine	——

TABLE XIII

TEST OF "INTERFERENCE" BY LAPINISED VIRUS.

Cattle were injected with lapinised virus and challenged at 48, 84 and 108 hours P.I.

Cattle No.	*Virus injected on 11.8.49*	*Date Challenged.*	*Hours between injection and challenge.*	*Reaction to challenge.*	*Result.*
8400	0·004 g. K. 15	13.8.49	48	Reacted	No protection at 48 hours.
8405	0·004 g. K. 15	15.8.49	84	Nil	Protection at 84 hours.
8398	0·004 g. K. 15	16.8.49	108	Nil	Protection at 108 hours.
8408	Control. Nil	18.8.49	——	Reacted	——
8403	0·004 g. K. 18	13.8.49	48	Reacted	No protection at 48 hours.
8411	0·004 g. K. 18	15.8.49	84	Reacted	No protection at 84 hours.
8412	0·004 g. K. 18	16.8.49	108	Nil	Protection at 108 hours.
8409	Control. Nil	18.8.49	——	Reacted	——

tion of the bovine virus to an extent whereby it would cause the death of the animal. This property of goat-adapted virus was very effective in the field in cutting short an outbreak in herds of cattle, individuals of which were infected with rinderpest. (Daubney, 1948; Edwards, 1949). If lapinised virus had the same power, then it would be effective in affording protection to grade and other susceptible cattle in circumstances when goat-adapted virus was unsuitable.

Eight cattle were divided into two groups of four. Three animals of each group were injected with 0·004 g. of lapinised virus and the fourth was left as an uninoculated control in each group. At 48, 84 and 108 hours after injection, one animal from each group was challenged and on the fifth day the control animals were challenged. In the first group, resistance to virulent virus was evident by the 84th hour; in the second by the 108th hour. (Table XIII.)

As all the inoculated controls reacted, it was concluded that cattle were protected 5 days after injection with lapinised virus. This result, while not as good as the protection afforded by goat-adapted virus, was a definite improvement on formolised-spleen vaccine. A field experiment, recorded elsewhere, gives additional support to the evidence presented above. (Brotherston, 1951.) Until the anti-viral bodies in the sera of cattle inoculated with lapinised virus can be measured, it will not be possible to determine whether this protection is due to "interference", or to the rapid production of antibody provoked by the virus material injected.

DISCUSSION

Rinderpest has proved to be adaptable to animals other than those usually regarded as natural hosts of the virus. Edwards (1927) made the first step towards the efficient control of rinderpest by the use of an attenuated virus, when he reported successful serial passage of the virus in goats and in rabbits. This goat-adapted virus work was exploited further by Stirling (1932, 1933), Saunders and Ayyar (1936), Pfaff (1938) and Daubney and Hudson (1937-1949).

Rinderpest has since been adapted to the rabbit (Nakamura, Wagatuma and Fukusho, 1938, quoted by Cheng and Fischman, 1948) to the developing hen's egg (Shope, Griffiths and Jenkins, 1946) and possibly to the guinea-pig (Baker, Terrence and Greig, 1946). The lapinised virus of rinderpest has been adapted to the egg (Walker, 1947; Nakamura, 1950) and to the pig (Hudson and Wongsongsarn, 1950).

In all the experimental animals in which rinderpest has been propagated, the presence of the virus can only be positively demonstrated by a temperature reaction, by the sub-inoculation of material into susceptible cattle and finally by challenge of such

cattle with virulent rinderpest virus. Infection of the rabbit with a fully lapinised rinderpest virus, however, results in a disease syndrome and characteristic macroscopic lesions can be identified. This may prove to be of great value in the future; serum-neutralisation investigations for the presence of antibodies in the sera of cattle may now be attempted, using the rabbit as a "live indicator" for the presence of lapinised virus.

The virus of rinderpest appears to be versatile in that virus-mutants have been induced to propagate in several species of experimental animals. Should the smaller laboratory animals (for example, mice) be found to act as hosts, experiments would be facilitated in that large numbers of animals could be used and the statistical significance of results would be enhanced.

It is hoped that this line of investigation will be pursued and that small laboratory animals may eventually exhibit a recognisable disease syndrome after continuous passage of the virus of rinderpest.

SUMMARY

The findings of Cheng and Fischman (1948) have been confirmed at Kabete. The Nakamura strain III of lapinised rinderpest virus was also "fixed" for the local breeds of rabbits and during 21 passages at Kabete there was no increase in virulence for rabbits, or attenuation of the antigenic properties for cattle.

Freeze-drying of the major virus-containing tissues of rabbits ensured the maintenance of the virus and dried material or "seed-virus" remained viable for long periods when kept under optimum conditions.

The symptoms and post-mortem findings of infected rabbits at Kabete have closely simulated those reported by other workers. It was noted that the temperatures of normal rabbits kept under laboratory conditions were slightly lower than those generally accepted. Rabbits below the age of $4\frac{1}{2}$ months did not react thermally when injected with the virus, but showed lesions typical of rinderpest; pregnant does were also unsuitable subjects for the propagation of the virus.

Alternate passage of the virus in cattle and rabbits appeared to alter the character of the virus, which showed a decreased virulence for rabbits with an irregular and somewhat increased virulence for cattle.

Co-habitation of infected rabbits with non-infected rabbits and infected cattle with susceptible cattle, did not result in passage of the virus by contact to the susceptible animals.

In contra-distinction to goat-adapted virus, lapinised virus provoked a very mild reaction in high and medium grade cattle at Kabete which could not be readily recognised. The development of an immunity in cattle so inoculated could not be determined until challenged with virulent rinderpest virus. The im-

munity induced by the injection of lapinised virus in susceptible cattle was sufficient to prevent clinical symptoms of rinderpest after challenge with Kabete bovine virus.

Lapinised virus induced a serviceable protection in cattle challenged 84 hours and at 108 hours respectively, after inoculation.

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LAPINISED RINDERPEST VIRUS AND A VACCINE: SOME OBSERVATIONS IN EAST AFRICA

II. Field Trials with Lapinised Vaccine

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INTRODUCTION

In East Africa, three general methods are used to protect cattle against rinderpest:—(*a*) vaccination with goat-adapted virus, (*b*) vaccination with inactivated tissue vaccine, (*c*) inoculation of hyper-immune serum.

Goat-adapted virus. This attenuated virus is used to immunise cattle of indigenous breeds which are more resistant to rinderpest than those of the European breeds. The protection conferred is serviceable within 24 to 72 hours (Pfaff, 1938, Daubney and Hudson, 1939), and is considered to last from four to six years, (Haddow, 1938, Minett, 1940.) Under normal environmental conditions, the reaction induced by goat-adapted virus may cause severe loss in condition, followed by a mortality of up to two per cent. (Daubney, 1948); under adverse conditions and in susceptible cattle the loss may be as high as 20 per cent.

Inactivated-tissue vaccines. In Kenya, a formalin-inactivated spleen vaccine is used and the accepted method requires three inoculations at weekly intervals, with an annual supplementary inoculation to re-inforce immunity. It is used to protect pure-bred, grade and highly susceptible indigenous cattle. The resulting immunity is good but does not begin until about ten days following the commencement of the series of injections, and in animals being immunised for the first time is not absolute until after the third weekly injection. This delay is a serious disadvantage when rinderpest threatens grade stock. The inactivated spleen vaccine is also expensive to prepare.

Hyper-immune serum. This method is seldom used as the immunity conferred is transient, the serum is expensive and it precludes the immediate use of other more permanent methods.

While the mainstay of control and eradication of rinderpest in East Africa will most likely be goat-adapted virus, there are certain classes of stock in which it is not possible to use this vaccine. There are also circumstances in which goat-adapted virus is not suitable, because of the comparatively severe reaction which it provokes. A cheap vaccine which would confer a rapid immunity in highly susceptible cattle would be preferable to inactivated-tissue vaccine and would be of particular benefit to the dairy farmer. Reports of the properties of lapinised rinderpest virus indicated that a vaccine prepared from it might solve the difficulties which have been mentioned briefly.

This paper records field trials with such a vaccine and confirms the findings of the workers in China. Cheng and Fischman (1948) used a vaccine in the field with apparent success although no challenges of the inoculated cattle were reported. It is fortunate that at Kabete there are facilities for challenging cattle vaccinated in field trials, and also for following the histories of animals in which the vaccine was used.

GENERAL EXPLANATION

After one initial trial by the writer, freeze-dried vaccine was sent for field trials to Kenya, Tanganyika and Uganda. It was decided to obtain representative numbers of vaccinated cattle, and, when possible, to have them sent to the laboratory for challenge with virulent bovine virus. Unfortunately, it was not always possible to obtain consent to leave uninoculated cattle with the experimental cattle to act as controls for the possible presence of natural rinderpest. Where permission was so obtained, a few controls only were kept, as the value of cattle at the time was such that owners were reluctant to risk any possible loss.

Vaccine was prepared in the usual way. The tissues from infected rabbits killed between the 60th and the 72nd hour after injection were suspended in five times the weight of infected blood, freeze-dried and dispensed in vacuum-tight vials in quantities of 0·25 g. or 0·5 g. Titrations in cattle were carried out at the laboratory before issue and each dose was so calculated that each animal inoculated in the field would receive twenty minimum protective doses. For example, if cattle titrations indicated that 1 g. of dried vaccine contained 2,000 doses, then 50 cattle were to be inoculated, each with 2 ml., after reconstituting 0·5 g. in 100 ml. of sterile saline. The inclusion of an uninoculated control ox with the cattle in which each batch of vaccine was tested served as a safety precaution; in the unlikely event of an infectious agent contaminating the vaccine, the control animal would serve as an indicator. In addition, each batch of vaccine was tested by the intra-peritoneal injection of a sample into 2 guinea-pigs and 4 mice.

The introduction of lapinised vaccine was quite new to the field staff in the early days of the trials, so that when officers could not be "briefed" personally, full instructions were sent out by letter. At this time there was no evidence regarding the stability or viability of the virus under varied field conditions, so that the need to observe the following precautions was emphasized. Tubes of vaccine should be kept on ice at all times prior to use and must be used before the expiry date. Only sufficient vaccine should be reconstituted for use within half an hour. During that time the container was to be kept cold, preferably on ice, and out of direct sunlight. Syringes should be cool before being charged and should not be left half-full while awaiting presentation of cattle. Cattle were to be adequately identified by brands, and information was

to be sent to the laboratory concerning the numbers inoculated, the breed of cattle, relevant dates of inoculation and the previous anti-rinderpest inoculation history. Cattle which had recently received serum or spleen vaccine were not recommended for inoculation with lapinised virus vaccine at this time. Farmers were to be encouraged to sell cattle and uninoculated controls to the laboratory at a later date so that the state of immunity could be checked by challenge with live bovine virus. If this was not possible, a percentage of cattle inoculated were to be challenged with goat-adapted virus and the results sent to the laboratory.

FIELD TRIALS IN KENYA

Pilot Experiment

In March 1949, the European Settlement Board breeding-farm requested the Department of Veterinary Services to protect their stock in view of the proximity of the farm to the Masai Reserve in which there was an outbreak of rinderpest. As goat-adapted virus was not entirely suitable for the grade young-stock and the in-calf animals, the opportunity was taken to employ lapinised virus and to leave uninoculated animals as controls. The stock consisted of some 700 head of cattle disposed as follows: —100 unweaned calves, 200 weaned calves, 200 in-calf heifers, 200 cows with the unweaned calves at foot and a few native bulls and work-oxen. The cows were of the Serenli breed from the Juba River of Somalia; the calves and the heifers were the progeny resulting from the artificial insemination of the Serenli cows with Jersey and Guernsey semen issued by the Central Artificial Insemination Station. There were also 70 native Serenli calves. Cattle were grazed in separate herds as grouped above.

On 8th March, 1949, the cows and in-calf heifers were inoculated with dried vaccine batch, K. 11. The unweaned calves and weaners were each divided into two groups one of which was given fresh virus vaccine and the other dried virus vaccine of the same batch (K. 11). Uninoculated controls were left in each of the groups.

Vaccine from passage K. 11 had been prepared and dried at the laboratory on the previous day, but about 10 g. of the suspension was stored on ice, so that fresh material could be used in some animals, with the object of assessing the usefulness of this product and comparing the immunity which it conferred with that of the dried vaccine. Fresh material was injected subcutaneously as 2 ml. of a dilution of 1 g. of emulsion in 100 ml. of sterile normal saline. Dried vaccine was injected as 2 ml. of 0·5 g. in 100 ml. of saline. At the inoculation crushes, containers of reconstituted material were kept on ice and sufficient was prepared to inoculate 50 head at one time. Prepared fluid was discarded if presentation of cattle was delayed unduly. Cattle in the groups were given an appropriate hair brand to distinguish them and all uninoculated controls were branded with the figure "O."

For ten days following the inoculations the temperatures of 20 animals from each group were taken morning and evening; there were no significant elevations in the representative cattle. The staff of the station had instructions to inform the laboratory of any unusual rise in temperature or sickness, but none was reported. On the 10th day after inoculation 12 cattle were brought to the laboratory for challenge. Table I shows the history of cattle from each group (excluding the cows and heifers in-calf), which were removed to Kabete and challenged with bovine virus. It was not possible to obtain animals from the in-calf heifer or cow group for challenge. All the weaned cattle resisted bovine virus, except No. 3 which showed a slight thermal reaction on the third day after inoculation, thereafter the animal appeared normal; all the calves resisted challenge. Uninoculated weaner and calf controls proved susceptible, while virus controls reacted satisfactorily.

It was concluded that the vaccine had immunised the cattle and that it had not provoked a thermal response. As a large number of animals in various stages of pregnancy were inoculated without ill-effect, it appeared that the vaccine could be used with safety to protect in-calf cattle. Fresh vaccine conferred as good an immunity as the dried vaccine but differences experienced during the inoculation of the cattle led to a decision not to use the fresh preparation in the future.

Duration of Immunity. Challenge by Inoculation

Having vaccinated many cattle of suitable age at the European Settlement Board Farm, it was intended to challenge the immunity of groups of animals at intervals during the ensuing two years. It was unfortunate that in July 1949, as a result of the low rainfall, food shortage became so acute that it was necessary to disperse the cattle. Nevertheless, fourteen weaned Serenli heifers, which as weaned calves had received dried virus K. 11 on the 8th March, 1949, were purchased before the dispersal. Table II shows the results of challenge of these cattle at Kabete 8½ months and 11 months after immunisation. At Kabete the heifers were kept in an isolated paddock.

After 8½ months four heifers out of four proved immune; one (No. 8201) showed a slight thermal reaction but otherwise remained normal. An uninoculated control (No. 8849) from the farm proved fully susceptible to bovine virus. After 11 months, two heifers out of four proved partially immune and two fully susceptible.

It was concluded that the vaccine had protected the animals for 8½ months but that the immunity had waned by the 11th month. In the laboratory, as the virus had been tested in cattle of much higher grade and hence greater susceptibility, it was possible that the mild lapinised virus was unable to provoke such a lasting immunity in the native stock which we obtained for challenge.

TABLE I

CHALLENGE ON 25.3.49 OF ANIMALS VACCINATED ON 8.3.49.

No.	*Description of Animal.*	*Type of Vaccine Used.*	*Reaction to Bovine Virus Challenge.*	*Result.*
1	Grade Weaner	Fresh	Nil	Immune.
2	X-bred Weaner	Fresh	Nil. Died 18 days after challenge, from extraneous causes	Immune.
3	X-bred Weaner	Dried	Slight temperature, no other symptoms	Immune.
4	X-bred Weaner	Nil	Control, reacted severely, but survived	Susceptible.
5	X-bred Weaner	Dried	Nil	Immune.
6	X-bred Weaner	Dried	Nil	Immune.
7	Grade Weaner	Nil	Control, reacted severely, died	Susceptible.
7029	Virus Control	Nil	Reacted, killed for vaccine	Susceptible.
7030	Virus Control	Nil	Reacted, killed for vaccine	Susceptible.
8	X-bred calf	Dried	Nil	Immune.
9	X-bred calf	Fresh	Nil	Immune.
10	Zebu calf	Nil	Control, reacted severely	Susceptible.
11	X-bred calf	Fresh	Nil	Immune.
12	X-bred calf	Dried	Nil	Immune.

TABLE II

CHALLENGE OF SERENLI HEIFERS 8½ AND 11 MONTHS AFTER VACCINATION.

No.	*Date of Challenge.*	*Reaction.*	*Result.*
8196	24.11.49	Nil	Immune.
8202	,,	Slight temperature, no other symptoms	Immune.
8203	,,	Nil	Immune.
8201	,,	Very slight temperature, no other symptoms ...	Immune.
8849	,,	Uninoculated control. Reacted	Susceptible.
8885	,,	Virus control. Reacted	Susceptible.
9166	9.2.50	Slight temperature, no other symptoms	Immune.
9165	,,	,, ,, ,, ,, ,, ...	Immune.
9164	,,	Reacted severely, killed for vaccine	Susceptible.
9163	,,	,, ,, ,, ,, ,, ...	Susceptible.

Nevertheless, as challenge by injection was a severe test of immunity, it was resolved to expose the remaining six heifers to contact with cattle reacting to rinderpest.

Duration of Immunity. Challenge by Contact

As indicated in the previous paper (Brotherston, 1951), page 286, it was resolved to expose cattle to contact with a more virulent strain of virus and the opportunity now occurred to carry this out. It was suspected that after 3 decades of "needle passage" the ability of the Kabete "O" strain to set up infection by contact might have been reduced. An experiment was, therefore, undertaken to subject the six heifers to prolonged exposure to the "O" strain. Should infection fail to become established, then the animals were to be exposed to contact with a virulent field strain.

Kabete "O" virus. Six heifers injected with lapinised virus K. 11 on 8th March, 1949 and four uninoculated susceptible cattle were placed in contact with animals used for the routine production of rinderpest tissue-vaccine on 22nd April. The vaccine-producers infected with Kabete bovine virus reacted typically and were killed on the fifth day after inoculation. The experimental cattle remained normal. On 29th April another group of cattle infected with Kabete virus was introduced; these animals also reacted satisfactorily and were killed five days later. By the 9th of May the six heifers and four controls had failed to show temperature reactions or other symptoms suggestive of rinderpest after a contact with Kabete "O" virus lasting 18 days, during which time no disinfection of the stable was carried out. For a subsequent experiment they were divided into three groups as follows:— Group A, heifers Nos. 8194, 8195 and 8197; Group B, heifers Nos. 8134, 8199 and 8200; Group C, uninoculated susceptible cattle Nos. 9436, 9459, 9461 and 9463 (Table III).

Virulent field virus. Group A heifers were stabled together with an animal (No. 9518) which was reacting typically to a virulent field strain of rinderpest isolated from Maralal. This animal had shown a temperature rise two days before the heifers were placed in contact and died from rinderpest three days after the heifers were introduced. The heifers remained in the stable for a further 13 days. No. 8194 first showed a temperature rise six days after the commencement of contact and exhibited mild symptoms of rinderpest for one week, after which it gradually returned to normal. Nos. 8195 and 8197 remained normal.

Group C susceptible cattle were placed in contact with ox No. 9519 which was also reacting typically to the virulent virus from Maralal. All four animals contracted rinderpest commencing on the 8th day following contact; two were destroyed, one died and one recovered after a severe illness.

Group B heifers were inoculated with Maralal virus. Two failed to show any reaction while one (No. 8134) reacted and died from typical rinderpest within seven days.

TABLE III

CHALLENGE BY CONTACT OF HEIFERS 13 MONTHS AFTER VACCINATION.

No.	*Group.*	*Contact with Kabete "0" Virus.*	*Contact with Field Virus ex-Maralal.*	*Inoculation with Field Virus ex-Maralal.*	*Result.*
8195	Group A Heifers	No reaction	No reaction	————	Immune.
8197		„ „	„ „	————	Immune.
8194		„ „	Mild reaction, recovered.	————	Partial Immune.
9436	Group C Uninoculated Cattle.	No reaction	Reacted rinderpest ...	————	Susceptible.
9461		„ „	„ „ ...	————	Susceptible.
9463		„ „	„ „ ...	————	Susceptible.
9459		„ „	„ „ ...	————	Susceptible.
8199	Group B Heifers	No reaction	————	No reaction	Immune.
8200		„ „	————	„ „	Immune.
8134		„ „	————	Died rinderpest ...	Susceptible.

———— signifies not carried out.

It has been pointed out (page 282) that the initial exposure of the heifers and the 4 unvaccinated cattle to Kabete "O" virus tended to obscure the latter part of the experiment. As the 4 unvaccinated cattle failed to react but later succumbed to the exposure to field virus, it was assumed that the Kabete "O" virus had lost the power to infect by contact and that the 6 vaccinated heifers were unaffected by the same exposure to Kabete "O" virus.

The fact that the vaccinated heifers of Group A were exposed to one reacting animal while the unvaccinated cattle of Group C were exposed to another ox reacting to field rinderpest virus, could be justifiably criticised in that the degree of exposure was not the same in both groups. However, because heifer No. 8194 contracted mild rinderpest while the other two remained normal and as all the unvaccinated cattle contracted typical rinderpest, it seemed reasonable to assume that cattle of both groups were exposed to contact with virulent infective material in some degree. The severity of exposure to contact infection in nature may be varied by many factors, while challenge by injection of rinderpest material can be controlled. The conditions of exposure in this case were kept as equal as possible but when reviewed at the end of the experiment were by no means ideal, for it was obvious that both groups should have been exposed to reacting virus-donors at the same time and in the same box. Nevertheless, it is felt that there are reasonable grounds for assuming that in Group A, 2 heifers were immune 13 months post-vaccination with lapinised vaccine and that one had retained a serviceable immunity after exposure to an animal reacting to virulent rinderpest; in Group B, two heifers were immune and one was susceptible after challenge by injection of virulent field virus.

Field Trials Thomson's Falls

In April 1949, 618 low-grade cattle on three separate farms were vaccinated with virus vaccine K. 19. The result of challenge at the laboratory of six of these cattle (8890, 8891, 9161, 9162, 9159, 9160) eight months later was disappointing in that two of the six proved to be susceptible. Rinderpest was present in an area close to these cattle at the time of vaccination, but extension of disease to the farms did not occur (Table IV).

In May 1949, 190 cattle on farm "M" in the same area were inoculated with virus-vaccine K. 15. Rinderpest was not actually present in cattle but game on the farm were dying of the disease and continued to do so up to July. Rinderpest among cattle existed on two neighbouring farms, but on farm "M" no losses were experienced. Ten vaccinated steers were brought to the laboratory in August 1950 (Table IV); all resisted challenge and thus proved immune 15 months after immunisation. It is recognised that these animals may have acquired a reinforced immunity by contact with rinderpest, but since none of the cattle on the

TABLE IV

CHALLENGE OF CATTLE FROM THE THOMSON'S FALLS AREA, KENYA.

Cattle No.	*Date of Vaccination and batch number.*	*Date of Challenge.*	*Time between vaccination and challenge.*	*Reaction.*	*Result.*
8890	16.4.49	12.11.49	7 months	Slight temperature, no other symptoms	Immune.
8891	16.4.49	12.11.49	7 months	Slight temperature, no other symptoms	Immune.
8825	Virus control	17.11.49	———	Reacted severely, killed for vaccine	———
9161	12.4.49	29.12.49	8 months	Nil	Immune.
9162	12.4.49	29.12.49	8 months	Reacted, killed for vaccine	Susceptible.
9159	12.4.49	29.12.49	8 months	Reacted, killed for vaccine	Susceptible.
9160	12.4.49	29.12.49	8 months	Nil	Immune.
8975	Virus control	29.12.49	———	Reacted, killed for Vaccine	———
391	24.5.49	31.8.50	15 months	Nil	Immune.
392	24.5.49	31.8.50	15 months	Nil	Immune.
393	24.5.49	31.8.50	15 months	Nil.	Immune.
394	24.5.49	31.8.50	15 months	Nil.	Immune.
395	24.5.59	31.8.50	15 months	Nil.	Immune.
396	24.5.49	31.8.50	15 months	Nil.	Immune.
397	24.5.49	31.8.50	15 months	Nil.	Immune.
398	24.5.49	31.8.50	15 months	Nil.	Immune.
399	24.5.49	31.8.50	15 months	Nil.	Immune.
400	24.5.49	31.8.50	15 months	Nil.	Immune.
425	Virus control	31.8.50	15 months	Reacted, killed for vaccine	———
426	Virus control	31.8.50	15 months	Reacted, killed for vaccine	———

farm were noticed to be infected at the time, it is reasonable to suppose that they were protected by the lapinised vaccine.

Mortality in Grade Herds

In September 1950, rinderpest was confirmed on a farm of high grade Herefords within 25 miles of the laboratory. The case has been reported elsewhere (Brotherston, 1951), but a brief mention of the circumstances and the results of using lapinised virus to combat rinderpest in infected herds of susceptible grade cattle may serve to emphasise another aspect of the protective properties of the vaccine. Two groups of the cattle, comprising adult steers and calves, were badly infected when control measures were instituted. In spite of the use of massive doses of hyper-immune serum and goat-adapted virus the mortality in these groups was high. Other herds were in contact with the infected animals, and cattle in the early stages of rinderpest were found in all except two. At this stage all the animals were given lapinised virus and Table V indicates the result of vaccination. Out of a total of 1886 animals the mortality was 196 (10·3 per cent.), whereas in cattle given lapinised virus it was 50 (3·1 per cent.). Milk records show that the yield was not materially affected by the use of the vaccine.

Lapinised Virus to Replace the Annual Inoculation of Cattle with Formolised Spleen Vaccine

A communication on this subject is in preparation by the author in conjunction with Dr. H. S. Purchase. Briefly, lapinised virus as an annual inoculation for the prevention of rinderpest appeared to have many advantages over the method of spleen vaccination. An experiment was, therefore, designed to discover if, in cattle which had previously received that vaccine, lapinised virus could be substituted for the annual reinoculation of spleen vaccine. The results of challenge of cattle at the laboratory indicated that such a procedure was possible. Fourteen cattle from a herd which had received a triple course of formolised spleen vaccine in August 1949 were challenged in November 1950 with bovine virus. Ten reacted severely, one died of rinderpest and three reacted mildly. Thirteen cattle of the same age and group from the same herd, given lapinised virus in October 1950, were challenged in November 1950; twelve remained normal while one only reacted very mildly and recovered with no symptoms other than a rise in temperature.

FIELD TRIALS IN UGANDA

In May 1949, a request for lapinised virus-vaccine was received from Uganda in order to control an outbreak of rinderpest in the Lango district. It was not advisable to use goat-adapted virus as cattle trypanosomiasis was endemic in the area and a large number of chronic cases existed. One thousand doses of lapinised vaccine, batches K. 20 and K. 21, were sent to the Veterinary Officer of the district; these batches, in doses of 0·004 g., had proved potent on

TABLE V.

MORTALITY IN GRADE HERDS INFECTED WITH RINDERPEST.

Herd.	*Treatment.*	*Total before Outbreak.*	*Deaths.*	*Remarks.*
Foster Herd 1	Lapinised virus	237	Nil.	Uninfected before treatment with vaccine.
Foster Herd 2	Lapinised virus	368	Nil.	
In-calf Herd	Lapinised virus	140	Nil.	Infected before treatment.
Dry Herd	Lapinised virus	157	Nil.	
Dairy Herd	Lapinised virus	209	22	
Adult Steers	Serum. Goat-virus ...	156	74	Disease first diagnosed in these animals.
Calves	Serum	101	72	
Oxen	Lapinised virus	17	1	Infected before Treatment.
Bulls (pure-bred)	Lapinised virus	12	4	
Young heifers and steers ...	Lapinised virus	434	20	
Heifers with the bull	Lapinised virus	55	3	
		1886	196	

TABLE VI

CHALLENGE OF UGANDA CATTLE.

No.	*Reaction to challenge.*	*Result.*	*Remarks.*
1	Nil	Immune	Vaccinated on 24th May, 1949, and challenged with goat-adapted virus on 29th July, 1949, 66 days later.
2	Nil	Immune	
3	Nil	Immune	
4	Nil	Immune	
5	Nil	Immune	
6	Nil	Immune	
7	Nil	Immune	
8	Nil	Immune	
8987	Nil	Immune	Vaccinated on 24th May, 1949, and challenged at Kabete with bovine virus on 9th December, 1949.
8988	Nil	Immune	
8989	Slight temperature only	Immune	
8990	Nil	Immune	
8991	Nil	Immune	
8992	Nil	Immune	
8993	Nil	Immune	
8979	Reacted	Susceptible	Kabete virus control.
8980	Reacted	Susceptible	Kabete virus control.

23rd April, 1949, and were to be used at 0·04 g. per dose. The vaccine was used under our instructions on May 24th, 1949. On September 2nd, 1949 the Director of Veterinary Services, Uganda, forwarded a copy of the results of a challenge test which had been carried out with goat-adapted virus on eight of the cattle by Mr. J. MacDonald of the Uganda Veterinary Department. It had not been practicable at the time of vaccination with lapinised virus to include susceptible uninoculated controls in the experiment, but a letter from the Director on September 29th confirmed that the outbreak of rinderpest had been suppressed and that no mortality as a result of lapinised virus vaccination had been recorded. In December, 1949, seven cattle from the District were sent to Kabete and challenged. One animal showed a rise of 102° F. in the morning and 104° F. in the evening of one day; otherwise all seven showed no symptoms of malaise. It was concluded that the lapinised virus induced a satisfactory herd immunity and that this was present 6 months after immunisation. (Table VI).

FIELD TRIALS IN TANGANYIKA

Lapinised vaccine, K. 16 C, was sent to Tanganyika and was used to inoculate 40 head of Ankole cattle at Bukoba on the 30th November, 1949. Ankole cattle are so highly susceptible to rinderpest that the general use of goat-adapted virus in this breed is impossible. The late A. R. Green, Veterinary Officer, Lake Province, carried out the first experiments, the others were completed by his colleagues after his death. Green (1950) observed that, after inoculation, the majority of Ankole cattle showed a marked thermal reaction, accompanied in some cases by inappetence and diarrhoea; uninoculated cattle did not show such symptoms. These observations were contrary to experience at Kabete with grade and cross-bred cattle, but were compatible with the hyper-susceptibility of Ankole cattle. On the 11th January, 1950, Green challenged five of the vaccinated cattle with goat-adapted virus and recorded that no obvious thermal reactions occurred but that three animals showed mild diarrhoea and inappetence. He was unable to challenge uninoculated controls at this stage, in view of the undesirable effect that severe reactions in the cattle would have had on the native owners (Table VII).

On 21st February, 1950, 12 vaccinated cattle were selected. Ten were challenged with goat-adapted virus and two were observed as temperature controls. One susceptible, uninoculated Ankole bull was also challenged as a virus control. No obvious reactions were recorded in the protected cattle except for one animal which had slight diarrhoea for two days. The control bull reacted strongly, showing general malaise, diarrhoea and small ulcers on the gums and on the tongue; the animal eventually recovered (Table VIII). Further observations by Green were prevented by his untimely death.

TABLE VII

RESULTS OF VACCINATION OF ANKOLE CATTLE WITH LAPINISED VIRUS AND CHALLENGE 42 DAYS LATER WITH GOAT-ADAPTED VIRUS.

Cattle No.	* *Reaction to lapinised virus 30th November, 1949*	*Reaction to challenge with Goat-adapted virus 11th January, 1950*	*Result*
05	Temperature rose to 103 F. ...	No rise of temperature—diarrhoea day 5 P.I.	Immune
30	Temperature rose to 103·8 F. ...	Nil	Immune.
73	No obvious rise in temperature...	No rise of temperature—diarrhoea days 3—5 P.I.	Immune.
35	Temperature rose to 103·4 F. ...	No rise of temperature—diarrhoea days 2—5 P.I.	Immune.
3	No obvious rise in temperature...	Nil	Immune.
06	Temperature rose to 105·8 F. ...	Not challenged. Temperature normal	———
5	Temperature 104 F.	Not challenged. Temperature normal	———
53	Temperature rose to 103·8 F. ...	Not challenged. Temperature normal	———

* All cattle showed dullness, loss of appetite and general symptoms of malaise between days 4 and 10 P.I.

Eight cattle, vaccinated with K. 16 C on 30th November, 1949, were challenged with goat-adapted virus on 30th August, 1950, nine months after immunisation. Three aditional animals were observed as temperature controls and one uninoculated bull was challenged as a virus control. Symptoms did not appear in the eight animals except that No. 37 was off its food for two days. The virus control bull reacted strongly (Milne and Woulfe, 1950). It was concluded that after 9 months the immunity induced by the lapinised vaccine was still present. Table VIII shows the results of this experiment.

TABLE VIII

CHALLENGE OF ANKOLE CATTLE WITH GOAT-ADAPTED VIRUS AT 83 DAYS, AND AT 10 MONTHS AFTER VACCINATION WITH LAPINISED VIRUS.

Cattle No.	*Reaction to challenge on 21.2.50 (83 days).*	*Result.*
5	Nil	Immune.
63	Nil	Immune.
67	Nil	Immune.
7N	No temperature, diarrhoea 4 and 5 days	Immune.
7T	Nil	Immune.
06	Nil	Immune.
0T	Nil	Immune.
57	Nil	Immune.
76	Nil	Immune.
55	Not challenged, temperature normal	———
6T	Not challenged, temperature normal	———
KAJU	Virus control. Reacted	Susceptible.

Cattle No.	*Reaction to challenge on 31.8.50 (9 months).*	*Result.*
00	Nil	Immune.
33	Nil	Immune.
0	Nil	Immune.
37	No temperature—off food	Immune.
53	Nil	Immune.
67	Nil	Immune.
No number	Nil	Immune.
C 1	Not challenged, temperature normal	———
C 2	Not challenged, temperature normal	———
C 3	Not challenged, temperature normal	———
Bull	Virus control. Reacted	Susceptible.

DISCUSSION

The good results obtained during trials in the field indicated that in lapinised vaccine the Veterinary Services had obtained another weapon with which to combat rinderpest. Because of the mild reactions provoked by the vaccine in highly susceptible cattle, it is visualised that it will become the method of choice to immunise those cattle which are at present protected by inactivated-tissue vaccine. Daubney (1928) introduced inactivated-tissue vaccine as a safe method of protecting highly susceptible cattle against rinderpest in Kenya. This method, combined with the use of goat-adapted virus in Zebu stock, enabled the Veterinary Services for all

practical purposes to suppress rinderpest in most of the areas. At the present time, the popularity of pure-bred cattle is on the increase, and there are now large numbers of grade crosses of European breeds with Zebu cattle. All this stock must be protected against rinderpest and it is considered that the lapinised vaccine has the following advantages. Vaccine from the rabbit is not expensive to prepare; one injection only is required to give protection; the antigenic powers of the vaccine can be estimated quickly and accurately in cattle; it is safe, having no adverse effect on pregnant cattle or on the milk-yield of cows; immunity is rapidly conferred, so that vaccine may be used to combat outbreaks of rinderpest; many thousands of doses may be packed in a small container.

It is true that the vaccine provokes little visible reaction and induces a comparatively short period of immunity, but it is suggested that these disadvantages are outweighed by the advantages.

With regard to Ankole cattle, lapinised vaccine appears to provoke a definite reaction. It is tentatively suggested that future experience may show that, in these cattle, the protection afforded by lapinised vaccine may prove to be of longer duration than one year. In certain areas where cattle trypanosomiasis is endemic, the use of goat-adapted virus is undesirable, even in Zebu cattle, as it causes a severe reaction with a consequent lowering of resistance, so that the chronic disease flares up; in such circumstances lapinised vaccine will prove invaluable.

It has already been indicated that lapinised vaccine may be used to replace the annual reinoculation in cattle protected with inactivated-tissue vaccine. Before the lapinised vaccine method is brought into general use, however, it will be necessary to show that the immunity engendered by a primary injection can be effectively reinforced by a second inoculation given one year later.

SUMMARY

In East Africa, freeze-dried, lapinised virus vaccine was used successfully for the control of rinderpest in the field. These results confirm the findings of Cheng and Fischman (1948) in China.

Indigenous Zebu cattle, high and low-grade crosses of European breeds with Zebu cattle, pure-bred European cattle and hypersusceptible Ankole cattle were successfully immunised. Of these, only the Ankole cattle exhibited a marked clinical reaction as a result of inoculation with lapinised virus.

When vaccinated cattle were challenged by the subcutaneous injection of virulent rinderpest, the duration of the immunity was shown to last for between 8 and 15 months. When tested by exposure to cattle infected with rinderpest, the immunity lasted 13 months.

The injection of lapinised virus had no adverse effect on pregnant cattle, and did not reduce the yield of cows in milk.

One experiment indicated that lapinised virus could be used for the reimmunisation of cattle which had received a triple course of formolised-spleen vaccine one year previously.

Many thousands of cattle have been vaccinated with lapinised virus but so far comparatively few have been obtained for immunity tests at the laboratory. However, when lapinised virus has been used in Kenya, in areas where natural rinderpest was present, it has not once failed to protect the cattle. On one occasion, in a herd of 1886 grade cattle some of which were infected with rinderpest, vaccination curtailed extension of the disease and considerably reduced fatalities.

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THE PATHOGENESIS OF RABIES FOLLOWING INOCULATION INTO THE MASSETER MUSCLE

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INTRODUCTION

Goodpasture (1925) having satisfied himself that "when a virulent neurotropic strain of the virus of herpes simplex is inoculated in sufficient quantity into the right masseter muscle of rabbits, one can demonstrate the first resulting cerebral lesion within the corresponding fifth motor nucleus", thought it might be possible to "demonstrate the first lesions of rabies in the ganglion cells of the fifth motor nucleus on the same side, if one inoculated the virus into a masseter muscle". From such experiments he hoped to adduce further evidence of axonal transmission of a neurotropic virus. Since the conclusions reached by Goodpasture and Teague (1923) and Goodpasture (1925) with regard to herpes are in disagreement with those of Marinesco and Dragenesco (1923, 1932), Pette (1931) and Field (1951, a) it was thought advisable to examine the earliest lesions which follow inoculation of the masseter with rabies virus.

MATERIAL AND METHODS

The animal used throughout has been the rabbit and the virus the Pasteur strain of fixed rabies (kindly supplied by Professor Lépine). Passage brain was stored at −24 or −40 degrees Centigrade without glycerine. A one in ten suspension in saline was made by grinding with sterile quartz sand and one millilitre of the supernatant after spinning for one minute at 3,000 revolutions used as inoculum. This was injected into the masseter muscle in three or four directions without withdrawing the needle. Animals were sacrificed at the earliest elevation of temperature or at the onset of symptoms. Histological investigation of the pons and medulla as well as other parts of the nervous system and the viscera was carried out in all thirteen rabbits of the series.

RESULTS

The temperature became elevated as a rule after six or seven days and the first nervous signs generally followed in 24 to 48 hours. In no instance was it possible to demonstrate early paralysis of the masticatory muscles on the side of the inoculation. All animals were able to bite well on an object introduced into the mouth, though it did seem on palpation that the inoculated masseter muscle contracted less forcibly than that of the opposite side. The site of initial paralysis is shown in Table I.

Table I.

Both hind limbs	E. 290*	E. 303	E. 304	E. 350	E. 351	
Contralateral hind limb	E. 285	E. 293				
Both fore limbs	E. 284**					
All four limbs	E. 291					
Contralateral fore and hind limbs ...	E. 292					
Ipsilateral fore and hind limbs ...	E. 354					

* More marked on inoculated than on opposite side.
** More marked on opposite than on inoculated side.

Two animals were sacrificed before the onset of paralysis.

No significant naked-eye changes were found at post-mortem examination.

HISTOLOGICAL EXAMINATION

Masseter

The site of inoculation showed little abnormality. Some muscle fibres were hyalinised and took on a uniform eosin staining, whilst here and there were islands of muscle fibres containing centrally placed nuclei. Such foci showed a moderate infiltration with lymphocytes. Gram's staining revealed no organisms. No infiltration of intramuscular nerve filaments was present.

Cervical lymph nodes.

These presented no abnormality in two cases in which they were examined.

Gasserian ganglion of the inoculated side

This was severely affected in all cases, practically all ganglion cells showing severe chromatolysis. Many nuclei were dense, shrunken and crenated with a perinuclear halo. Capsular cell increase was pronounced and Babès nodules frequent. Islands of lymphocytes were common (Fig. 1). Nerve fibres above and below the ganglion appeared normal and in particular the Redlich-Obersteiner interval showed no infiltration. No Negri bodies were present.

Pons and medulla

Changes within the pons were in all cases minimal. There was often a widespread lysis of Nissl substance and sometimes this might be localized to one part of the cytoplasm. Many nuclei were darkly staining and sometimes presented the granular degeneration described by Schaffer (1890, 1931). Vacuolation of the ground substance was widespread in many cases and similar to that observed in herpetic encephalitis (Field, 1951, a). Some ganglion cells showed well marked incrustation of the Golgi network. Such features seemed to be especially prominent in Deiters' nuclei. *In no case was there a special or localised lesion in the motor nucleus of the fifth nerve of the inoculated side.* Perivascular

cuffing was very slight, an occasional vessel—especially a dilated vein—showing a single or at most a partial double row of mononuclear cells. Such cuffing was most marked near the floor of the fourth ventricle. (Figs. 2, 3.)

Brain at level of tuber cinereum, cervical and lumbar cord

Apart from an occasional minor degree of chromatolysis (which is difficult to assess) nothing abnormal was found. One case showed marked vacuolation in the cord.

Contralateral Gasserian ganglion

In all cases this showed changes identical with those in the ganglion of the inoculated side (Fig 4).

Spinal dorsal root ganglion

In the *cervical region* the second and eighth ganglia were examined and found to contain severe lesions. The nerve cells showed severe chromatolysis, eccentricity of nucleus, nuclear shrinkage and crenation with perinuclear halo formation. The cell bodies were occasionally vacuolated. These changes were of about equal intensity on both sides (Figs. 5, 6). In some cases where the spinal cord was sectioned with ganglia attached the contrast between the severely affected ganglia and normal cord was striking.

The *thoracic ganglia* showed some degree of infiltration though much less than in the cervical region. The infiltration was patchy and relatively few Babès nodules were present. Changes were of about equal intensity on both sides.

The *lumbar ganglia* showed only slight changes, and these were of the types seen in the ganglia higher up. In E.370 which was an early case showing fever, excitement and general incoordination with a tendency to deviate to the side of the inoculation in hopping, the lumbar ganglia were apparently quite normal. In other rather more advanced cases a minor degree of swelling of capsular cells was present. Babès nodules were encountered only once in the lumbar ganglia.

Superior cervical sympathetic ganglion

In three cases the ganglion of the inoculated side showed lesions. In two of these definite foci of small round cells were present (Fig. 7) whilst in the third there was a localized group of shrunken and chromatolytic ganglion cells without infiltration.

Vagus ganglion

In two cases in which these were examined they were found to show severe lesions with Babès nodule formation.

DISCUSSION

Goodpasture (1925) using street rabies injected 9 rabbits and allowed five to live long enough to show definite physical signs. Of these five with symptoms of nervous system involvement, three

showed weakness of the forelimbs on the inoculated side first, in one case the opposite forelimb was first affected and in one both fore limbs simultaneously. No hind leg paralysis was observed in any of the five animals with nervous involvement but one of the non-paralysed animals showed "general loss of muscular co-ordination". He concluded therefore, that "following an inoculation into the right masseter muscle the anterior extremities are first affected and more often on the right side". The present series (using fixed virus) has given different results.

The findings set out above show clearly that after inoculation of the masseter muscle the earliest histological changes are to be found in the Gasserian ganglion of the same and the opposite side as well as in other dorsal root ganglia. Moreover, the ganglia furthest removed from the segmental level of the inoculation are the least affected. The motor nucleus of the fifth nerve is intact in every case. Goodpasture, who was working with street rabies, came to the conclusion that it was "not possible to prove that Negri bodies appear first in the ganglion cells of the fifth right motor nucleus following the inoculation of the virus of rabies into the masseter muscle on the same side" (p. 555), and further that there was "no conclusive evidence that the virus enters the brain through nerves" (p. 556). Nevertheless he was inclined to believe that the clinical course of the disease could be best explained on the theory of neural transmission of the virus from the site of inoculation.

In assessing this conclusion the following considerations should be borne in mind.

1. The absence of early paralysis of the masticatory muscles following inoculation of the masseter.

2. The high proportion of cases (five out of thirteen) in which paralysis appeared first in the hind limbs. There is here a divergence from the results obtained by Goodpasture (see above). He seemed to imply (p. 556) that the occurrence of paralysis in the forelimb of the inoculated side in three out of five cases could be taken as evidence that the virus reached the brain through the fifth nerve and then proceeded down the cord. Rabies, however, like poliomyelitis shows a strong tendency to produce paralysis in the hind limbs even when inoculated intracerebrally (Babès, 1912), the forelimbs becoming affected later. Moreover, a successful intravenous inoculation commonly leads to initial posterior paralysis (Pasteur, Chamberland, Roux and Thuillier, 1882), so that the finding of an early posterior paralysis in the present series of experiments is consonant with virus reaching the brain through the fifth nerve or by the blood stream. Whilst it is widely accepted following the early work of Schaffer (1890) that the onset of paralysis in human rabies is related to the part bitten, some notable exceptions have been recorded. Thus Marie and Chatelin (1919) describe the case of a child of eleven bitten on the right upper lip by a dog. No pain or discomfort of any sort was experienced in

the wound which healed rapidly, but five weeks later an acute illness developed beginning with strange nocturnal attacks of unrest. Soon both legs became paralysed whilst the face and arms remained unaffected. Later the arms, too, were involved, the whole picture resembling an acute poliomyelitis of Landry type similar to that met with in the rabbit.

3. The early infiltration of dorsal root ganglia. The lesion in the ipsilateral Gasserian ganglion was, as expected, severe. What was not expected was the occurrence of an equally intense change in the opposite ganglion (the intervening pons being almost normal) and the lesions of the other root ganglia. Dorsal root ganglion changes in rabies have, of course, long been known (Nepveu, 1872) and were fully described by van Gehuchten and Nellis (1900), though their early occurrence has not been emphasized. Goodpasture (1925) did not examine the ganglia of his early cases. The introductory symptoms in human cases sometimes suggest dorsal root involvement not only in the territory bitten but more widely. Schaffer (1890) gives protocols of six cases in five of which a detailed history was obtained. His case 5 complained initially of pains of root character in the bitten arm and these soon spread to the opposite temple and ear region as well as to the chest wall on the side of the bite. These symptoms were especially marked at the onset of the rapidly fatal illness and were accompanied by generalized increase in reflex excitability.

Following peripheral introduction virus might reach the dorsal root ganglia, other than those in neural relationship with the part inoculated, either by the blood stream or through the cerebrospinal fluid. The ganglia are relatively easily accessible to certain colloidal dyestuffs (e.g. trypan blue) introduced into the blood stream and there is evidence that peripheral irritation enhances this accessibility in diminishing degree as one passes away from the level of the irritation (Zakaraja, 1932). Whilst rabies virus has been but rarely demonstrated in the blood (Marie, 1905; Marie and Urbain, 1931) the fact that it may pass from an infected mother to the foetus (Perroncito and Carita, 1887; Konradi, 1905) indicates that infection by the blood stream is possible even though no virus may be demonstrable therein by the methods commonly in use. A somewhat similar state of affairs occurs in herpes. Thus Mariani (1923) found that if a blister was raised on the skin of an animal inoculated upon the cornea with herpes virus, the latter could be demonstrated within the blister fluid. Demonstration of herpes virus in the blood of such animals by ordinary methods is, however, exceptional (Levaditi, 1926).

Whilst the cerebrospinal fluid is generally held to be non-infective, there have been some reports to the contrary (Konradi, 1922). It is possible that here again the ordinary methods for demonstration of the virus are not adequate for the absence of virus from the cerebrospinal fluid is remarkable. Particles of

Indian ink (0·5 to 1·5μ) introduced into the subarachnoid space of the rabbit soon collect in the arachnoid cul-de-sacs around the spinal nerve roots, especially in the lumbar region, and may be found within the dorsal ganglia where they are taken up by certain of the capsular cells (Field and Brierley, 1948; Brierley, 1950). It is probable that particles of fixed rabies virus which have a size of from 100 to 150$m.\mu$ (Galloway and Elford, 1936) to 140 to 210$m.\mu$ (Levaditi *et al*, 1936) could take a similar course. This possibility is supported by the observation that herpes virus introduced into the cerebrospinal fluid of the rabbit produces lesions in the dorsal root ganglia over the same territory as that reached by India ink under like conditions (Field, 1951, a). Several workers have suggested on the basis of detailed histological examination of the nervous system that the cerebrospinal fluid is the medium by which virus invasion actually takes place (Schukru and Spatz, 1926; Seifrid and Spatz, 1930) and this has been supported by the experimental work of Speransky (1927).

A parallel would seem to exist between the present findings of early lesions in the dorsal root ganglia and those of Flexner, Clark and Amoss (1914) in poliomyelitis. These workers recorded that "monkeys in the preparalytic stage of infection, before any detectable weakness of muscles has set in but while in the state of excitement, already show lesions of the intervertebral ganglia" (p. 207). McClure (1943) found that 2 per cent of the spinal ganglia of "sick" monkeys showed lesions, and that this proportion was significantly increased when poliomyelitis virus was administered either by stomach tube or by enema, the animals being sacrificed before the onset of clinical signs of infection. Early changes in the spinal ganglia have also been recorded by Faber, Silverberg and Dong (1950) following the casual exposure of apparently healthy monkeys to poliomyelitis infection. To what extent such changes are specific remains to be determined, for minor degrees have been produced in intraneural injection of weak formalin (Suslow, 1932) and it may well be that further study will show intervertebral ganglion infiltration to be part of a defence mechanism. Unfortunately it was not possible in the present work to titrate the virus content of the various spinal ganglia.

In conclusion it may be stated that the present experimental results, like those of Goodpasture (1925), offer no support for belief in the axonal transmission of rabies virus from the inoculated masseter muscle to the pons. They suggest rather that infection is disseminated either by the blood stream or by the cerebrospinal fluid. It must, however, always be borne in mind that we may be dealing with some process not explicable on the basis of spread of an infective agent from the inoculated site, but more of the nature of a general reaction on the part of the organism to the entry of a special irritant (virus) into contact with its nervous system.

CONCLUSIONS

Inoculation of the masseter muscle of the rabbit with fixed rabies virus does not lead to an early paralysis of the masticatory muscles of the same side. In five out of thirteen animals paralysis began in the hind limbs.

Severe lesions occur in the dorsal root ganglia of both sides and are much less intense in the lumbar than in the cervical region.

No lesion was found in the corresponding motor nucleus of the fifth nerve even when there was already evidence of widespread involvement of the nervous system.

Spread of the blood stream and cerebrospinal fluid is briefly considered.

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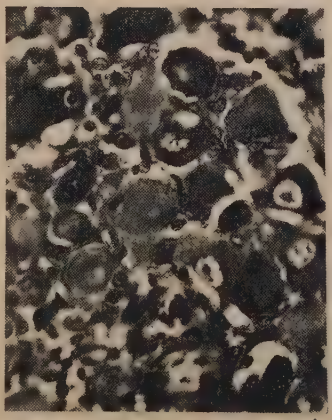

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Fig. 1. Right Gasserian ganglion (E. 285) ; animal sacrificed on 16th day at first signs of weakness in left leg following inoculation of right masseter. Degenerative changes in ganglion cells. invasion by capsular cells and infiltration with lymphocytes. Haematoxylin and Eosin. × 400.

Fig. 2. Floor of fourth ventricle (E. 291) showing perivascular cuffing and vacuolation of ground substance. Haematoxylin and Eosin. × 35.

Fig. 3. High power view showing slight degree of the cuffing. Haematoxylin and Eosin. × 540.

Fig. 4. Left Gasserian ganglion (E. 285)—Degenerating changes in ganglion cells and infiltration. Haematoxylin and Eosin. × 330.

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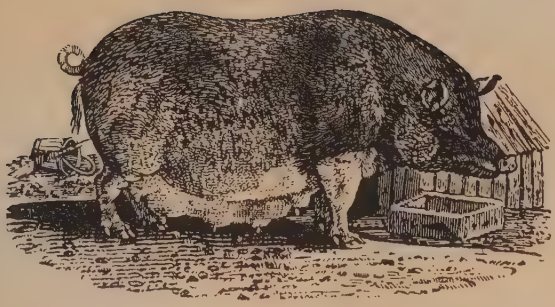
Fig. 5. Right 8th cervical dorsal root ganglion (E. 285) showing degenerative changes in ganglion cells and lymphocytic infiltration. Haematoxylin and Eosin. × 65.

Fig. 6. Left 8th Cervical dorsal root ganglion (E. 285)—to be compared with fig. 5. Haematoxylin and Eosin. × 65.

Fig. 7. Right cervical sympathetic ganglion showing severe lesions. Haematoxylin and Eosin. × 118.



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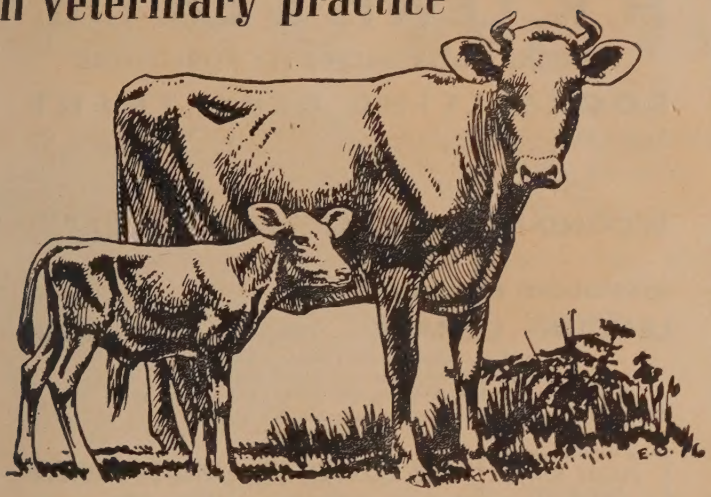

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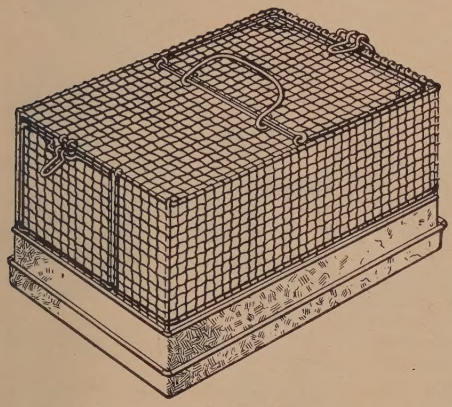

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